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CHEMICAL ANALYSIS

OF

HEALTHY AND DISEASED URINE,

QUALITATIVE AND QUANTITATIVE.

BY

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PREFACE.

In the preparation of this Manual no labor has been spared to adapt it to the requirements of all persons into whose hands it may fall. With this object in view, simple qualitative tests are given in full, and the rationale of chemical processes fully explained, and, to meet the requirements of students somewhat familiar with laboratory work, nearly all the methods employed in quantitative estimations are given in full; especially is this true in processes peculiar to work in physiological chemistry.

The plan of the part of the work devoted to the Qualitative Examination of the Urine is understood by examination of the Index of Chapters. Chapter VI is devoted to Processes of Examination of the Urine and Sediments, and Chapter VII is given to the consideration of Concretions and Stones. The preceding chapters, I to V inclusive, are devoted to the Physical and Chemical Properties of Constituents of the Urine. These chapters, therefore, embrace materials with which the student should become quite familiar by experimentation, in order that the work laid out in Chapters VI and VII may not be mechanical.

In Chapter VIII is found a brief consideration of Processes and Apparatus employed in Quantitative work, and also the Preparation of Normal Solutions required. In this connection the student should not infer that a general work on Quantitative Analysis, embracing details of manipulation with description of apparatus, is not required. The most important facts, as regards laboratory work, have been incorporated, that they may be of some assistance to those who have no complete treatise at hand.

In Chapters IX, X and XI are found Methods for Estimation of Quantities of Constituents of Healthy or Diseased Urine, as the case may be, and in Chapter XII are Methods for Estimation of Quantities of Albuminous Bodies, and Sugar in Urine. As an aid to students to gain an insight into the constitution of food and urine, and the relationship they sustain to each other, the four Tables in the Appendix are introduced. Tables 2, 3 and 4 are from Zuelzer, while Table 1 was arranged from data collected by the author.

The Quantitative part of the work is perhaps fuller than is generally required, but with a moment's consideration it is understood that it was by quantitative analysis that nearly all the facts concerning the transformation of tissue and the elements of food have been brought to light; and that quantitative analysis will eventually be employed in many cases as an aid in diagnosis and in the treatment of disease, is an assured fact if the practice of medicine is to become to a certain extent fixed, as mathematics and physics.

Presumably it would not be speculative to assume that the natural history of a specific disease embraces a typical composition of the urine. In this direction much is due to the labors of Zuelzer, of Germany, and Lépine, of France. By Zuelzer the quantity of nitrogen excreted by the kidneys is placed at 100, and in health the variation of the relative quantity of each constituent of the urine is known from the results of numerous estimations. This basis of measurement is satisfactory in all cases, as nitrogen is a constituent of all tissues, and nitrogenous products are excreted by the kidneys. When an organ or particular tissue yields an increased quantity of products of waste these products are, to a great extent, found in the urine, in which case the relative quantities of these products are increased. Without some standard of measurement, the fact that a product, P_2O_5 for example, is found in increased quantity in the urine, is of little or

no value; but if the quantity of P_2O_5 is much greater than in health, while that of nitrogen is not greatly increased, attention is directed to tissues containing much phosphorus, as lecithin, in nerve centres. From these facts it is implied that the estimation of the quantity of nitrogen in urine should be made, when the quantities of other constituents are determined.

Until recently this was an obstacle not easily overcome, when the only methods for the estimation of nitrogen leading to correct results were those of Dumas and Varrentrapp and Will, but now, as Kjeldahl's method is known to yield results as accurate and not requiring much time or apparatus, there is no reason why the employment of quantitative estimations will not lead to a knowledge of types in the constitution of the urine peculiar to different diseases. It was with this view that the quantitative part of the work was made somewhat lengthy, and that the methods employed are the most exact.

VAN NÜYS.

INDIANA UNIVERSITY CHEMICAL LABORATORY,
BLOOMINGTON, IND., December, 1887.

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ERRATUM.

On page 63, 12 lines from top, "crystals of hæmatin" should read "crystals of *hæmin*."

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CHEMICAL ANALYSIS

OF

THE URINE.

CHAPTER I.

Properties of the Urine—Nitrogenous Bodies, including Oxalic and Benzoic Acids—Urea—Fermentation—The Xanthin Group of Compounds—Uric Acid—Kreatinin—Hippuric Acid—Indican—Urobilin—Oxalic Acid—Benzoic Acid.

PROPERTIES OF THE URINE.

Normal urine, when fresh, is generally clear, and after the lapse of a few hours a cloudy or flaky sediment in irregular masses is usually observed at the bottom of the vessel containing the urine. As urine generally holds in solution acid phosphates and sulphates it is usually acid in reaction, but it is sometimes neutral or alkaline, depending very much on the diet. If the urine is alkaline when fresh it is usually cloudy or opaque, as there are certain compounds of calcium and magnesium which are insoluble in alkaline fluids. The reason that different kinds of food affect the reaction of the urine is that in meat, eggs, cheese, etc., there are considerable quantities of phosphorus and sulphur and less of the metals of the alkalies—potassium and sodium. In the urine phosphorus and sulphur combined with oxygen and metals form acid salts if the metals are not in sufficient quantity to form basic salts. On the other hand, in vegetable food the quantity of potassium is greater, and by the ingestion of food largely vegetable in kind the blood becomes more alkaline in reaction than by the ingestion of animal food.

When normal urine is alkaline the urine formed subsequently is rendered acid by the ingestion of a dilute acid. No free acid, however, is formed in the urine, but the basic or neutral phosphates are changed to acid salts—

$$\underset{\text{Basic Phosphate.}}{Ca_3(PO_4)_2} + 4\,HCl = 2\,CaCl_2 + \underset{\text{Acid Phosphate.}}{CaH_4(PO_4)_2},$$

or

$$\underset{\text{Neutral Phosphate.}}{2\,CaHPO_4} + 2\,HCl = CaCl_2 + \underset{\text{Acid Phosphate.}}{CaH_4(PO_4)_2}.$$

If dilute acid be administered in excess to a carnivorous animal, an increased quantity of ammonia combined with acids is found in the urine. The ammonia is formed at the expense of the quantity of urea. After a hearty meal the urine is usually alkaline until the lapse of a few hours, when the urine formed is acid, unless the food is largely vegetable in kind. During the period of secretion of the gastric juice the blood becomes less alkaline as it loses chlorine, which leaves bases to form hydrochloric acid in the gastric juice; consequently, the urine becomes neutral or alkaline from the presence of the alkaline carbonates. Later in the period of digestion, when absorption and assimilation take place, this drain from the blood ceases, as the hydrochloric acid is, in part, absorbed, and part combines with the potassium and sodium of the biliary salts, and some of the phosphorus and sulphur of the food appear in the urine in acid combinations.

Normal urine varies greatly in color, from being nearly colorless to dark brown. Usually, however, it is of an amber or straw color. In disease, the urine is generally more highly colored than in health. Exceptions are in diabetes mellitus, chlorosis, anæmia, etc. In the febrile state the urine is highly colored, with high specific gravity, owing to increased quantity of urea. Coloring matters of the bile or blood may impart a deep color—yellow, red or dark brown—without there being an increased quantity of urea; or the urine may be of an abnormal color, due to the ingestion of rhubarb, tarry substances, etc., and the urine be normal in every other respect. As the color of normal urine varies greatly, so its odor is subject to variation. The chemical constitution of the body to which the urine owes its odor is not known. It volatilizes very slowly, as urine does not lose all of its odor by boiling or evaporating.

The specific gravity of the urine varies from 1002 to 1030;

that is, a volume of urine would weigh 1010 or 1025 grammes or grains, while an equal volume of water would weigh 1000 grammes or grains. The specific gravity of urine is lessened by drinking much water, while it is increased by a warm, dry atmosphere and consequent free perspiration. As activity of the skin is increased by exercise to exhaustion, so the specific gravity of the urine is likewise increased from the formation of an increased quantity of urea. On the other hand, by moderate exercise the specific gravity of the urine is not increased.

UREA.

Of the constituents of urine containing nitrogen, urea is the most important, as it constitutes nearly 3 per cent. of the weight of urine.

It is composed of carbon, hydrogen, oxygen and nitrogen and has the formula of

$$CH_4N_2O,$$

which group of elements has the structural formula of

$$CO\begin{matrix}\diagup NH_2 \\ \diagdown NH_2\end{matrix}.$$

It is a substitution compound of ammonia, NH_3, in which one atom of hydrogen is substituted by CO, as

$$\begin{matrix}NH_2 \diagdown \\ NH_2 \diagup\end{matrix} CO.$$

It is apparent that urea is an organic body, yet the structure of the molecule is comparatively simple, being very little more complex than that of an inorganic molecule. Urea is the principal decomposition product of muscular and other tissues. It is formed in the blood (Schmiedeberg) by the withdrawal of the elements of water from ammonium carbonate—

$$\underset{\text{Ammon. Carb.}}{(NH_4)_2CO_3} - 2H_2O = \underset{\text{Urea.}}{CH_4N_2O}.$$

46.66 per cent. of urea is nitrogen. The average quantity of urea excreted by the kidneys of an adult in twenty-four hours is 31 grammes.

One gramme of urea corresponds to 13.72 grammes muscular tissue. By consulting Table 1 in the Appendix it is seen that in 31 grammes urea there are 14.464 grammes nitrogen, and about 93 per cent. of the total quantity of nitrogen excreted by the kidneys is in this compound; but the quantity of urea

excreted in twenty-four hours is subject to variation, ranging from 25 to 40 grammes, depending not only on the quantity of the nitrogenous elements of food having entered the circulation, but on the rate of the désassimilation of tissues, and as the latter process is facilitated by fever, urea appears in increased quantity in the febrile state. In determining the rate of désassimilation of the tissues by the quantity of urea excreted, the kind and quantity of food ingested and the quantity of nitrogen in the fæces are taken into account; hence simple estimations of urea in the urine do not afford sufficient data, but in fevers there is wasting of the tissues, with a corresponding increase in quantity of urea excreted and diminished quantity of sodium chloride or chlorine in the urine. On the other hand, when there is no abnormal waste of tissues, but there is an increased quantity of urea excreted coming from the nitrogenous elements of food ingested, the quantity of chlorine (in combination) in the urine is likewise increased. In diabetes and anæmia the quantity of urea excreted is increased without fever. Urea is a colorless crystalline body ; the crystals contain no water of crystallization and undergo no change in the air. It is soluble in water and alcohol and the solution is neutral in reaction. At 130° urea melts, if free of water. It acts as a base, forming salts with acids. Urea can be separated from the urine by adding barium mixture (for the preparation of which refer to Chapter VIII) to the urine—one or two litres—until, after mixing well by stirring with a glass rod, a precipitate ceases to form. Filter, and evaporate the filtrate on a water bath to syrupy consistence. By standing twenty-four hours the urea will crystallize, when the mass is pressed between porous paper, transferred to a litre flask and treated with about 500 c.c. 90 per cent. alcohol, and after shaking more or less for thirty minutes, filter, and evaporate the filtrate on a water bath. The residue is nearly pure urea, but is highly colored by the coloring matter of the urine, to remove which dissolve in water and treat with a solution of potassium permanganate; filter, and evaporate the filtrate on a water bath to dryness.

FERMENTATION.

Normal urine, when exposed to the air at ordinary temperatures, undergoes remarkable changes: losing its acidity, it becomes turbid, emits the uriniferous odor, and when heated gives off

alkaline vapors. By microscopic examination the urine is found impregnated with bacteria, the *Micrococcus ureæ*. Besides bacteria, if the urine is strongly alkaline in reaction, crystals of magnesium, ammonium phosphate, ammonium urate, *a* and *b*, Fig. 29, page 83, and calcium oxalate, Fig. 16, page 74, are found in the sediment. There is in solution a large amount of ammonium carbonate, urea having been decomposed by absorbing water—

$$\underset{\text{Urea.}}{CH_4N_2O} + 2H_2O = \underset{\text{Ammon. Carb.}}{(NH_4)_2CO_3}.$$

That bacteria is the cause of the fermentation is shown by putting a small quantity of fermenting urine into fresh urine, when the latter will ferment much sooner than it otherwise would. It is, therefore, of great practical importance to put fresh urine, for analytical purposes, into clean vessels, and for closing bottles or flasks containing urine to employ corks which have not been in use for the same purpose. Obstinate diseases of the bladder are sometimes produced by introducing the micrococcus ureæ into the bladder by the use of catheters which had been used before, but not properly cleaned. In case of fermentation taking place in the bladder, the alkaline reaction of the urine is generally due to the formation of ammonium carbonate, but occasionally bacteria is found in fresh urine decidedly acid in reaction, the process not having continued long enough for the formation of sufficient ammonium carbonate to transform the acid salts to basic compounds.

Urine undergoing fermentation is turbid in every part, while urine alkaline from the presence of potassium and sodium carbonates, by transmitted light, appears more or less clear, with floating particles or suspended flakes.

To prevent urine from fermenting a few days, it is kept in bottles surrounded by ice, or, in the absence of which, it is rendered strongly acid with hydrochloric acid.

THE XANTHIN GROUP OF COMPOUNDS.

Sarkin or Hypoxanthin,	$C_5H_4N_4O$.
Xanthin,	$C_5H_4N_4O_2$.
Uric Acid,	$C_5H_4N_4O_3$.

The bodies here enumerated, with another member of the group, guanin—

$$C_5H_5N_5O,$$

are found in glandular and muscular tissues. It is seen by the formulæ of these bodies that they are closely related. That in all probability they have a common origin—products of the reduction of albuminous bodies—is rendered more evident by the fact that each has been produced from other members of the group by chemical processes. Xanthin is found in the urine in exceedingly small quantities. Refer to Table I in the Appendix. Whether sarkin is a constituent of normal urine is doubtful. A body resembling it in many respects is found in the urine, but in mere traces.

URIC ACID.

Although uric acid is found in human urine in small quantity, yet it is a constant constituent. In the urine of birds and reptiles it is in great quantity. 33.3 per cent. of uric acid is nitrogen. It is more complex in constitution than urea. Its formula is—

$$C_5H_4N_4O_3.$$

Usually uric acid is in combination in the urine, forming urates, yet it sometimes appears as a sediment. In twenty-four hours 0.2 to 1 grm. uric acid is excreted by the kidneys. The average quantity is about 0.6 grm. Consult Table I in the Appendix. When the food is composed principally of meats, eggs, etc., the quantity of uric acid excreted is increased. As the tissues are reduced or désassimilated in far greater quantity during fever than during health, the amount of uric acid excreted is likewise increased. In health the relative quantity of uric acid and urea is 1 to 51. In fever attended by checked aëration of the blood, as in pneumonia, the relative quantity of uric acid is increased. An increase of uric acid is likewise observed in leucocythæmia. In this disease aëration of the blood is diminished by reason of the diminution of the red corpuscles of the blood. In articular rheumatism the quantity of uric acid excreted is not increased; the sediment of the urates and uric acid, so often observed in this disease, results from concentration of the urine and strong acid reaction. In diabetes and advanced stages of Bright's disease the quantity of uric acid in the urine is diminished.

Uric acid is a crystalline body, rhombic and tabular in form. It is tasteless, odorless, and in cold water nearly insoluble. Uric

acid forms salts with potassium, sodium, or ammonium carbonate or hydrate, according to the equations—

$$C_5H_4N_4O_3 + 2NaOH = C_5H_2Na_2N_4O_3 + 2H_2O,$$
$$C_5H_4N_4O_3 + Na_2CO_3 = C_5H_3NaN_4O_3 + NaHCO_3.$$

From the formulæ of the urates formed by these reactions, it is seen that there are two different urates, one containing one atom of Na or K, and the other two atoms; consequently, uric acid is a dibasic acid, that is, it contains two atoms H, which may be substituted by two atoms of a monad metal. Urates containing but one atom of K, Na or NH_4 are acid urates, those containing two are neutral urates. There are also urates composed of a molecule acid urate, and a molecule uric acid, as—

$$C_5H_3KN_4O_3, C_5H_4N_4O_3.$$

The acid urates are frequently found as a sediment in urine of acid reaction. All urates are decomposed by dilute hydrochloric acid, with the liberation of uric acid—

$$C_5H_2Na_2N_4O_3 + 2HCl = 2NaCl + C_5H_4N_4O_3.$$

To separate uric acid from the urine, it is concentrated by evaporation to about one-half its volume, when it is rendered strongly acid with dilute hydrochloric acid, and, after standing twenty-four hours, crystals of uric acid are observed adhering to the walls of the glass containing the urine—sedimentum lateritium. By filtering and collecting the crystals on the filter and washing with some cold water, uric acid can be obtained nearly pure but highly colored. As the acid urate of ammonium is far less soluble in water than the corresponding urates of sodium or potassium, it forms when a solution of ammonium chloride is added to the urine, especially after the lapse of a few hours.

Besides the microscopic examination showing crystalline forms of uric acid and urates, Fig. 8, page 71, its presence can readily be determined by evaporating some strong nitric acid, mixed with uric acid on a watch glass, to dryness, and treating the residue with a small quantity of ammonia water, when a purple-red color is observed.

KREATININ.

Kreatinin, $C_4H_7N_3O$, and kreatin, $C_4H_9N_3O_2$, differ, as seen by their formulæ, the latter containing H_2O more. Both are constituents of urine in certain conditions, as these bodies change

into each other, according to the condition. In alkaline urine, kreatin is in greater quantity. In urine strongly acid, kreatinin is found with either no kreatin or a mere trace. Physiologically, they may be regarded as one body, but, as the urine is usually acid, kreatinin is considered the normal constituent.

Chemically, it is regarded as a substitution compound of ammonium, as it is a base, and, like ammonia, it forms salts by direct union with acids without the separation of water. In the urine of an adult secreted in twenty-four hours, kreatinin is in quantities from a trace to 1.3 grm.; the average is about 0.7 grm. In fevers it is increased in quantity, and in diabetes the quantity is diminished. To determine the presence of kreatinin in the urine (Neubauer and Salkowsky), 500 c.c. urine is treated with milk of lime until it becomes alkaline in reaction, when a solution of calcium chloride is added until a precipitate ceases to form, known by filtering a small quantity and testing the filtrate with a solution of calcium chloride, and if no precipitate forms, enough has been added. Calcium hydrate and chloride serve the purpose of separating the phosphoric acid. Filter, and render the filtrate distinctly acid with hydrochloric acid, avoiding an excess of acid. Evaporate nearly to dryness, and, when cold, treat with about 100 c.c. strong alcohol. Transfer the residue and fluid to a flask or beaker, render alkaline with a solution of sodium carbonate; after which render acid with acetic acid. The solution should be acid in reaction, but not with hydrochloric acid. After standing ten hours, filter, and to the filtrate add a strong alcohol solution of zinc chloride, to which some sodium acetate is added, if the solution is acid in reaction. Kreatinin zinc chloride will form. The formula is—

$$(C_4H_7N_3O)_2ZnCl_2.$$

Fig. 1.

The microscopic character of this body is shown by Fig. 1. If the urine contains sugar, it is decomposed by putting some yeast in it and keeping it at 25° to 30° twenty-four hours. Albuminous urine is rendered acid with dilute sulphuric acid, boiled twenty minutes and filtered before it is subjected to further treatment.

Kreatinin, when in aqueous solution, imparts the alkaline reaction. With acids it combines, forming salts. When a solution

of sodium nitroprussiate is added to a dilute solution of kreatinin (Weyl's test), and then sodium hydrate is added, the solution changes to a red color, which, by standing, turns yellow. This is the most delicate test for kreatinin in urine. The presence of sugar or albumen in the urine does not interfere with the reaction. Kreatinin reduces CuO in Fehling's solution to Cu_2O.

HIPPURIC ACID.

In the urine of herbivorous animals hippuric acid is found in considerable quantities. In human urine the quantity is less. In the urine formed in twenty-four hours by a man of average weight there is 0.15 to 0.6 grm. hippuric acid. The average quantity is 0.4 grm. The quantity of hippuric acid excreted by the kidneys depends very much on the kind of food, yet with a nitrogenous (animal) diet the acid does not disappear from the urine. It has been found that when albumen is decomposed by means of the pancreatic ferment, phenylpropionic acid is formed, and, by oxidation, this acid forms benzoic acid, which, by uniting with glycocol, produces hippuric acid. The following equations will serve to illustrate some of the chemical changes which take place in the formation of benzoic and hippuric acids:—

$$\underset{\text{Phenylpropionic Acid.}}{C_6H_5 - CH_2 - CH_2 - COOH} + 6O = 2H_2O + 2CO_2 + \underset{\text{Benzoic Acid.}}{C_6H_5 - COOH}.$$

$$\underset{\text{Benzoic Acid.}}{C_6H_5 - COOH} + \underset{\text{Glycocol.}}{CH_2\begin{cases}NH_2\\COOH\end{cases}} = \underset{\text{Hippuric Acid.}}{CH_2\begin{cases}NH - C_6H_5 - CO\\COOH\end{cases}} + H_2O.$$

Glycocol, as well as phenylpropionic acid, is formed in the process of intestinal digestion. Phenylpropionic acid having entered the circulation is oxidized, forming benzoic acid as one of its products, when the latter acid, uniting with glycocol, forms hippuric acid with the separation of water. This is the source of hippuric acid in the urine when nitrogenous food is taken, but, as the quantity of the acid is greatly increased by a diet composed principally of vegetables, it cannot be considered the only source. As hippuric acid originates in different ways, it is necessary to take into account many data in determining if it arises from pathological processes. 7.87 per cent. hippuric acid is nitrogen. It is a crystalline body, usually crystallizing in four-sided prisms. The acid is colorless and has a bitter taste. It is not very soluble in cold water, but more soluble in hot water. In alcohol it is

soluble. Hippuric acid yields but few characteristic reactions, so that its separation from the urine is necessary before subjecting it to reactions. For this purpose about 1000 c.c. urine (Bunge and Schmiedeberg) are rendered alkaline with sodium carbonate, filtered, and the filtrate evaporated to near dryness, and the residue treated with alcohol in several portions of 50 c.c. and filtered each time. Evaporate the alcohol and treat the residue with a small quantity of water, transfer to a glass cylinder, acidify the mixture with hydrochloric acid and shake up five times with about 30 c.c. acetic ether, and each time, when the ether separates from the water, it is removed by means of a syphon or pipette. The acetic ether is washed by shaking it up with water in a glass cylinder, and drawing off the acetic ether as before. By evaporating the acetic ether to dryness at ordinary temperatures, crystals of hippuric acid will be found in the residue. To remove benzoic acid and any fat that may be present, dissolve the residue in water and transfer to a glass cylinder, shake up with benzole, and when it has separated from the water, remove by means of a syphon or pipette. By evaporating the aqueous solution, hippuric acid will separate in the form of crystals. Besides the crystalline forms of hippuric acid, Fig. 10, page 71, its presence is determined by treating it with strong nitric acid and evaporating on a water bath to dryness, when the residue is transferred to a dry test tube and carefully heated. If hippuric acid is present, a peculiar odor, resembling that of the oil of bitter almonds, is produced, due to the formation of nitrobenzole—$C_6H_5NO_2$.

INDICAN.

Indican is the potassium salt of a substitution compound of sulphuric acid, H_2SO_4, in which the group of elements C_8H_6N takes the place of one atom of hydrogen of H_2SO_4. The free acid, indoxylsulphuric acid,

$$C_8H_6N\diagdown\!\!\diagup H\diagdown\!\!\diagup SO_4,$$

is not stable, but its salts are well known. Indican is not a coloring matter, but when indoxylsulphuric acid is oxidized indigo blue is formed.

$$2C_8H_6N\diagdown\!\!\diagup H\diagdown\!\!\diagup SO_4 + O_2 = 2H_2SO_4 + C_{16}H_{10}N_2O_2.$$

Indoxylsulphuric Acid. Indigo Blue.

The formula of indol is C_8H_7N. It is formed in the intestines by the action of pancreatic juice and bacteria on albuminous bodies. Much of it passes off with the fæces, and that which is absorbed appears in the urine as indican. It is known that this is one source, if not the only one, of indican in the urine, for when indol is introduced into the blood, the quantity of indican in the urine is greatly increased. When the conditions for increased fermentation of the contents of the intestines are favorable, as in obstinate constipation and peritonitis, the quantity of indican in the urine is greatly increased, and when the quantity of albuminous food is reduced the amount of indican in the urine is likewise reduced. About 11 milligrammes indican is excreted by kidneys of a man of average weight in twenty-four hours. Indican is a solid, soluble in water and partly soluble in alcohol. Indigo blue when brought in contact with nascent hydrogen changes to indigo white—

$$\underset{\text{Indigo Blue.}}{C_{16}H_{10}N_2O_2} + H_2 = \underset{\text{Indigo White.}}{C_{16}H_{12}N_2O_2}.$$

Indigo blue is insoluble in water; soluble in alcohol, ether and chloroform. The test for indican in the urine is based on the fact that indigo blue is formed by the oxidation of indoxylsulphuric acid.

To one part urine (Jaffé's test) add one part hydrochloric acid, specific gravity 1.124. The acid serves the purpose of decomposing indican, liberating indoxylsulphuric acid—

$$\underset{\text{Indican.}}{C_8H_6\overset{K}{\diagdown\!\diagup}N\diagdown\!\diagup SO_4} + HCl = KCl + \underset{\text{Indoxylsulphuric Acid.}}{C_8H_6\overset{H}{\diagdown\!\diagup}N\diagdown\!\diagup SO_4}.$$

To the fluid add, drop by drop, a filtered solution of bleaching powder (1 part to 20 parts of water). Shake the mixture well after each addition of the solution, and when a decided change of color of the fluid takes place shake up with a few cubic centimetres of chloroform. The latter as it settles will be colored blue.

Besides indican there are in the urine, under certain circumstances, analogous compounds in which phenyl, C_6H_5, and cresyl, C_7H_7, are substituted for one atom of hydrogen of H_2SO_4. Refer to sulphuric acid, page 36.

UROBILIN.

The normal color of the urine is not due to any one coloring matter as found by spectroscopic examination. The most im-

portant coloring matter, however, is urobilin. The formula of urobilin is $C_{32}H_{40}N_4O_7$. It is a derivative of bilirubin, a constituent of bile. This body, by the action of nascent hydrogen, changes to urobilin—

$$\underset{\text{Bilirubin.}}{2C_{16}H_{18}N_2O_3} + H_2O + H_2 = \underset{\text{Urobilin.}}{C_{32}H_{40}N_4O_7}.$$

In the intestines nascent hydrogen is formed, and by it bilirubin of the bile is reduced to urobilin, and the latter is in part absorbed by the blood and excreted by the kidneys. There is reason to believe that urobilin is itself in part reduced by nascent hydrogen, from the fact that urine sometimes becomes more deeply colored by exposure to the air, by which a colorless body is oxidized to form urobilin. This has been more satisfactorily proven by separating the coloring matters from the urine by shaking with some basic lead acetate, filtering, and exposing the colorless filtrate to the air, when it may become colored. By spectroscopic examination of the filtrate, when colored by oxidation, urobilin is found. Urobilin is a dark, amorphous powder. It is nearly insoluble in water, soluble in alcohol, ether, and chloroform. In a solution of potassium, sodium, or ammonium hydrate it dissolves.

From alkaline solutions, rendered nearly neutral, it is precipitated by a salt of a heavy metal, for example, lead acetate, silver nitrate or copper sulphate. It is partly precipitated from alkaline solutions by rendering them acid. Solutions of urobilin, when examined by the spectroscope, exhibit a band between the lines b and F, Spectrum 4, Table 1, page 60. For spectroscopic examination, prepare a solution in ammonium hydrate to which some zinc chloride has been added. The band is brought out more distinctly by these solvents. To test for urobilin, the use of the spectroscope is more satisfactory than the employment of chemical tests. For description and use of the spectroscope refer to page 62. As there is a very small quantity of urobilin in normal urine, the urine of a person having fever is examined for urobilin. About 200 c.c. of the urine is treated with basic lead acetate, filtered, and the precipitate is washed once with cold water and dried at a low temperature. Triturate the dry mass in a mortar with strong alcohol (20–25 c.c.), to which add some dilute sulphuric acid to separate the lead, and after standing a few hours filter,

render the filtrate alkaline with ammonium hydrate, add a small quantity of a solution of zinc chloride and examine the solution with the spectroscope.

OXALIC ACID.

Oxalic acid combined with potassium and calcium is found in many vegetables. The formula of it is $C_2H_2O_4$. It is a dibasic acid soluble in water. The oxalates of potassium, sodium, and ammonium are soluble in water. Calcium oxalate is insoluble in water and dilute acetic acid, but soluble in water containing hydrochloric acid. Calcium oxalate crystallizes with one molecule water in tabular form and with three molecules water in octahedral forms; the latter usually form slowly, while the former results by the crystallization taking place in a short time. As normal urine is usually acid, calcium oxalate is held in solution. It is only when the urine becomes neutral or alkaline that it appears as a sediment. The changing of the urine from the acid to the neutral or alkaline condition is usually a slow process, so that calcium oxalate usually appears as a sediment in the form of octahedral crystals. For the character of the crystalline form refer to Fig. 16, page 74. To determine the presence of oxalic acid in the urine, about one litre urine is treated with an excess of a solution of calcium chloride and rendered alkaline with ammon. hydrate. After the lapse of a few hours the mixture is rendered acid with acetic acid, avoiding more than necessary. The acetic acid dissolves the phosphates of calcium and magnesium, but calcium oxalate remains undissolved. The urine is then filtered and the precipitate is washed with water, when it is dissolved on the filter with a small quantity of dilute hydrochloric acid, and after washing from the filter with some water the acid fluid is rendered alkaline with ammon. hydrate. The calcium oxalate will reprecipitate. After standing several hours, the precipitate may be examined by the microscope.

BENZOIC ACID.

As many kinds of vegetables, particularly fruits, contain small quantities of benzoic acid, it is introduced into the blood by employing a vegetable diet. But whether it is introduced with the food, or formed from albuminous bodies in the process of

intestinal digestion, it is to a great extent transformed into hippuric acid by uniting with glycocol. The conditions for the reactions to take place are present in the kidneys, as shown by the experiments of Bunge and Schmiedeberg. Refer to the reactions above, under hippuric acid. As some of the benzoic acid is liable to escape this reaction, it is sometimes found in normal urine. By the employment of the method of Bunge and Schmiedeberg for the separation of hippuric acid from the urine, benzoic acid is also separated. The method is given above. Benzoic acid is separated from hippuric acid with pure benzole. For this purpose shake the aqueous solution in a glass cylinder with benzole, and, when it has separated from the water, draw it off with a syphon or pipette. Repeat the process two or three times and concentrate the benzole solution by evaporation. If the acid is colored and otherwise impure, it is easily purified by dissolving the residue of the benzole with a small quantity of hot water, filtering, and crystallizing out by slow evaporation.

CHAPTER II.

Inorganic Constituents of Urine—Chlorine, Phosphoric Acid, Basic, Neutral and Acid Phosphates—Deportment of Acid Phosphates with Alkalies—Deportment of Soluble Phosphates with Soluble Salts of Calcium and Magnesium—Deportment of Neutral and Basic Phosphates with Acids—Glycerin-phosphoric Acid—Phosphorous Compounds in Healthy and Diseased Urine—Sulphur Compounds—Deportment of Sulphuric Acid as Sulphates and as Ester Compounds—Sulphur not as Sulphates and Esters—Origin of Sulphur Compounds in Urine—Carbonic Acid—Compounds of Calcium and Magnesium—Ammonia—Origin of Ammonia in Urine—Potassium and Sodium.

INORGANIC CONSTITUENTS OF URINE.

Inorganic bases in the urine are calcium, magnesium, potassium, and sodium oxides. Ammonia is also present, and is classified as an inorganic base. There is also a small quantity of iron oxide in normal urine, but the quantity is so small that it is not considered. Inorganic acids in the urine are hydrochloric, phosphoric, sulphuric, and carbonic acids. Silicic acid is found in traces in normal urine. Inorganic bodies are separated from the urine by evaporating and oxidizing the residue. For this purpose evaporate 100 c.c. urine to dryness in a porcelain dish, heat gradually over the free flame until a charred mass is formed and vapors are no longer given off. After cooling, treat with small quantities of water, decant into a small filter and preserve the filtrate. Dry the contents of the dish, heat to a high temperature until the carbon is oxidized, when cold treat with water, filter, and evaporate the filtrate in the evaporating dish to dryness. The carbon of the residue of urine, being intimately mixed with the soluble bodies of the ash, is difficult to oxidize, hence they are removed with water before the oxidation is completed. If some normal urine be evaporated slowly, the crystals first formed are nearly pure sodium chloride, NaCl, and by continuing the evaporation a compound of urea and sodium chloride will crystallize out. It is well known that there is much of the sodium and chlorine combined in the urine, there being besides sodium an insufficient quantity of metals, or bases, to combine with more than 30 per cent. of the chlorine. Compounds, which under ordinary conditions are insoluble in water, are in solution in the

urine, owing to the formation of acid salts; thus lime and magnesia, combined with phosphoric acid, are in solution in urine of acid reaction.

CHLORINE.

The quantity of chlorine in the urine is subject to variation, as the kidneys are not the only channel through which the chlorides are eliminated from the blood. The chlorides are found in perspiration, saliva, matters expectorated, bile, and the fæces. In health the quantity of chlorine in the urine depends largely on the amount of common salt taken with food, and also on the activity of the skin. Taking into account the amount of the chlorides taken with the food, the quantity of chlorine excreted by the kidneys during the forenoon is greater than in the same quantity of urine formed during the night. (Hegar.) By mental work the quantity of chlorine excreted is increased. During fever chlorine is found in the urine in less quantity than in health, but in convalesence from fever the quantity is generally increased, even if the diet remain the same. In pneumonia the quantity of chlorine in the urine is diminished, in diarrhœa the quantity is also diminished. In health the average quantity of chlorine excreted by the kidneys in twenty-four hours is 6 to 8 grms.

When a soluble chloride is brought in contact with a solution of silver nitrate, a white precipitate is formed, AgCl. It is insoluble in dilute nitric acid, but soluble in ammonium hydrate. To test for chlorine in the urine, acidify with dilute nitric acid, add solution of silver nitrate; a white precipitate will form.

PHOSPHORIC ACID IN COMBINATION AS PHOSPHATES.

In the urine there are different phosphates of the same metal, according as one, two, or all three of the atoms of H of H_3PO_4 (phosphoric acid) is substituted by the metal. The three compounds of sodium are NaH_2PO_4, Na_2HPO_4 and Na_3PO_4; the first is known as the acid, the second the neutral, and the third the basic, phosphate of sodium. The formulæ of the corresponding phosphates of calcium are—

$$\underset{\text{Acid Ph.}}{CaH_4(PO_4)_2}, \quad \underset{\text{Neutral Ph.}}{CaHPO_4} \quad \text{and} \quad \underset{\text{Basic Ph.}}{Ca_3(PO_4)_2}.$$

As a calcium or magnesium atom has twice the saturating power of a potassium or sodium atom, the formulæ differ. In

the urine phosphoric acid is partly combined with sodium and potassium and partly with calcium and magnesium. The former are generally known as the phosphates of the metals of the alkalies, or alkaline phosphates, and the latter as the phosphates of the metals of the alkaline earths. The phosphates of potassium and sodium, whether acid, neutral or basic, are soluble in water. The acid phosphates of these metals, in solution, change to neutral or basic phosphates when brought in contact with potassium or sodium hydrate—

$$NaH_2PO_4 + KOH = KNaHPO_4 + H_2O.$$
$$NaH_2PO_4 + 2NaOH = Na_3PO_4 + 2H_2O.$$

When neutral or basic alkaline phosphates are formed in the urine by the action of potassium or sodium hydrate, they react on the calcium and magnesium in the form of acid or neutral phosphates or other soluble salts, precipitating them as basic or neutral phosphates—

$$Na_2HPO_4 + CaCl_2 = CaHPO_4 + 2NaCl.$$
$$2Na_3PO_4 + 3MgHPO_4 = Mg_3(PO_4)_2 + 3Na_2HPO_4.$$

The acid phosphates of calcium and magnesium are quite soluble in water, imparting the acid reaction. They undergo no change by the addition of an acid. With a soluble calcium or magnesium salt they change to neutral or basic phosphates—

$$CaH_4(PO_4)_2 + CaCl_2 = 2CaHPO_4 + 2HCl.$$
$$MgH_4(PO_4)_2 + 2MgCl_2 = Mg_3(PO_4)_2 + 4HCl.$$

Generally there is not a sufficient quantity of calcium and magnesium to combine with more than one-third of the phosphoric acid to form basic salts, but when the phosphoric acid is in combination with calcium and magnesium, forming acid or neutral salts, as is generally the case in normal urine by rendering the urine alkaline with a solution of sodium or potassium hydrate, all of the calcium and magnesium are precipitated in the form of basic salts, and some of the phosphoric acid remains in solution combined with the sodium or potassium—

$$3CaH_4(PO_4)_2 + 12NaOH = Ca_3(PO_4)_2 + 4Na_3PO_4 + 12H_2O.$$

By filtering and adding to the filtrate a solution of calcium chloride, basic calcium phosphate precipitates—

$$2Na_3PO_4 + 3CaCl_2 = Ca_3(PO_4)_2 + 6NaCl.$$

As there are nearly two-thirds of the phosphoric acid in normal urine combined with the metals of the alkalies, the quantity of these phosphates is simply increased by rendering the urine alkaline with sodium or potassium hydrate. The neutral phosphates of calcium and magnesium are slightly soluble in water; the latter is more soluble than the former. With acids they change to acid phosphates—

$$2CaHPO_4 + 2HCl = CaH_4(PO_4)_2 + CaCl_2.$$

By boiling a saturated solution of either, the solution becomes turbid, owing to the formation of some basic phosphate—

$$4MgHPO_4 = Mg_3(PO_4)_2 + MgH_4(PO_4)_2.$$

By this reaction neutral urine becomes cloudy by boiling. The basic phosphates of calcium and magnesium are soluble in dilute acids, owing to the formation of acid phosphates—

$$Ca_3(PO_4)_2 + 4HCl = CaH_4(PO_4)_2 + 2CaCl_2.$$

Urine alkaline in reaction, is necessarily turbid, owing to the formation of basic phosphates of calcium and magnesium.

GLYCERIN-PHOSPHORIC ACID.

Besides the phosphates enumerated above there is a small quantity of glycerin-phosphoric acid in normal urine. As its name indicates, its component parts are glycerin, $C_3H_5(OH)_3$, and phosphoric acid, $PO\begin{matrix}\diagup OH\\ —OH.\\ \diagdown HO\end{matrix}$ The rational formula of glycerin-phosphoric acid is

$$\begin{matrix}\diagup OC_3H_5(OH)_2\\ PO—OH\\ \diagdown OH.\end{matrix}$$

It differs from soluble phosphates in not precipitating with magnesia mixture (for the preparation of which refer to Chapter VIII) and ammon. hydrate. Neither is the body precipitated from solution when a solution of calcium hydrate and chloride is added. By these reagents the phosphoric acid of the phosphates is separated. (Sotnischewsky's method.) Render the urine alkaline with the milk of lime, add a solution of calcium chloride until a precipitate ceases to form; filter, and evaporate the filtrate to dryness.

The residue contains the glycerin-phosphoric acid. Treat the

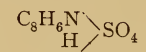
residue several times with alcohol, to dissolve out all bodies soluble in alcohol, when the residue is dissolved in water and treated with a small quantity of magnesia mixture and an excess of ammon. hydrate. By this reagent a small quantity of the phosphates, that may be in solution, is separated. After standing twenty-four hours, filter, and to decompose the glycerin-phosphoric acid in the filtrate, render strongly acid with sulphuric acid and boil fifteen minutes; after cooling, render the solution strongly alkaline with ammon. hydrate and add some magnesia mixture. Crystals of magnesium ammon. phosphate, $MgNH_4PO_4$, will separate. To prove that glycerin was in the combination with the phosphoric acid evaporate the filtrate to dryness, extract with alcohol, and evaporate the alcohol solution of glycerin to dryness, when the presence of glycerin in the residue is determined by placing some of it in a dry test tube, mixing well with some dry acid potassium sulphate, and applying heat. The penetrating odor of acrolein is evidence of the presence of glycerin. Moisten a borax bead on platinum wire with some of the residue and hold in the oxidizing zone of a Bunsen's flame; a green color imparted to the flame is characteristic of glycerin. The total quantity of P_2O_5 in the urine of an adult of twenty-four hours is 2.7 to 3.2 grms., but the quantity is subject to vary from 2.5 to 4.5 grms. The relative quantity of nitrogen placed at 100 is 18 to 20. From estimations of glycerin-phosphoric acid in normal urine, the quantity excreted in twenty-four hours is 0.030 to 0.060 grm. Occasionally, however, no glycerin-phosphoric acid is found in normal urine. By the ingestion of soluble phosphates with glycerin (Zuelzer) the quantity is increased to 0.354 grm. The quantity is also increased, according to Zuelzer, by narcosis of chloroform. The total quantity of phosphoric acid in the urine varies according to the kind of food ingested. By consulting Table 3 in the Appendix, it is found that the relative quantities of nitrogen and P_2O_5, in articles of food containing these bodies, differ. In brain, for example, the relative quantity of P_2O_5 (nitrogen 100) is 44, beef 12.8, etc. Zuelzer found that by the ingestion of different articles of food the relative quantity of P_2O_5 in the urine varies, as shown by Table 4 in the Appendix.

As brain substance contains lecithin, $C_{42}H_{84}NPO_9$, the quantity of P_2O_5 excreted by the kidneys is greatly increased by taking

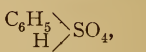
brain as food. The same investigator found that in depressed conditions of the nervous system, as during sleep or narcosis produced by chloroform, morphine or chloral, the quantity of P_2O_5 excreted by the kidneys is increased, and on the other hand the quantity excreted is diminished by moderate stimulation produced by alcohol or strychnin. In fever the relative quantity of P_2O_5 excreted by the kidneys is less than in health, but during convalescence it is greatly increased.

SULPHUR COMPOUNDS.

In the urine there are three classes of sulphur compounds: the sulphates, as Na_2SO_4 or $CaSO_4$; the ester compounds of H_2SO_4 in which certain organic radicals take the place of H in H_2SO_4, as


Indoxylsulphuric Acid.

and


Phenylsulphuric Acid.

and compounds the constitution of which is not fully known. These sulphur compounds are separated as they differ in their chemical properties. The sulphuric acid of the sulphates is separated from the urine by rendering it strongly acid with acetic acid, and on adding an excess of a solution of barium chloride, $BaSO_4$, will separate as a white precipitate. The acetic acid prevents the precipitation of barium phosphate and has no action on the second and third class of compounds, hence they remain in solution. The ester compounds of sulphur are decomposed by heating with hydrochloric acid, with the liberation of sulphuric acid. Separate the $BaSO_4$ from the solution by filtering, and render the filtrate acid with hydrochloric acid, boil a few minutes; a white precipitate, $BaSO_4$, forms, there being a sufficient quantity of barium chloride in the solution to combine with all of the sulphuric acid formed by the decomposition of the ester compounds. The filtrate of the second precipitation of barium sulphate contains the third class of sulphur compounds, the sulphur of which is oxidized to form sulphuric acid. For this purpose, concentrate the filtrate by evaporating in a platinum dish on a water bath, when the solution is rendered alkaline with a solution of sodium carbonate, after which add 7 grms. potassium nitrate for every 100 c.c. urine, evaporate to dryness, and heat the residue in the dish gradually until it fuses. After cooling, dissolve in

water, filter if necessary, render the solution acid with hydrochloric acid and evaporate to a small volume. If the solution is still acid transfer to a test tube, and test for sulphuric acid with a solution of barium chloride. By this process the sulphur is oxidized by the oxygen of the potassium nitrate to form sulphuric acid. The total quantity of sulphur, calculated as H_2SO_4, excreted by the kidneys in 24 hours is 2 to 4 grms., the relative quantity is 18 to 20 nitrogen placed at 100. The total quantity varies at different times, depending not only on the quantity of albuminous bodies and sulphates in the food, but on the condition of the liver. It was found by Zuelzer that when the bile is drawn off by establishing a fistula, less of the sulphur compounds are found in the urine. A few hours after a meal, the quantity of sulphur compounds in the urine increases, and preceding a meal, the quantity decreases. In the febrile state there is an increase in quantity, and during convalescence, the liver resuming its normal action, less of the sulphur compounds is found in the urine. The cause of an increased quantity of the sulphur compounds excreted by the kidneys when the liver is inactive, is that taurin, $C_2H_7NSO_3$, of the bile, having sulphur as a constituent, is not all absorbed by the blood, as a small quantity is found in the fæces. In convalescence the liver is more active, an increased quantity of bile passes into the intestines, and much of the taurin is excreted with the fæces. During digestion an increased quantity of bile is likewise formed, and as a result less sulphur is excreted by the kidneys. But as about 80 per cent. of the total quantity of sulphur excreted by the kidneys comes from albuminous bodies and sulphates of the food, as well as from the reduction of tissues, without first entering into the formation of taurin, slight changes in the physiological conditions of the liver bring about no variation in the quantity of sulphur compounds in the urine. The quantity of sulphuric acid, in the form of phenylsulphuric acid combined with sodium or potassium, is increased by the use of carbolic acid, whether used externally or internally, so that the urine, after having been rendered strongly acid with acetic acid, will yield no precipitate with a solution of barium chloride. The phenylsulphates are not poisonous, hence the test for sulphates with acetic acid and a solution of barium chloride has clinical importance in cases carbolic acid is used for;

as long as the urine forms a precipitate with these reagents, there is sufficient H_2SO_4 to form the phenylsulphates with carbolic acid, $C_6H_5(OH)$, thereby preventing the toxic effects of the latter.

CARBONIC ACID.

Normal urine contains carbonic acid, as acid or normal carbonates. Acid calcium or magnesium phosphate reacts on the carbonates, liberating carbonic acid gas—

$$CaH_4(PO_4)_2 + 2NaHCO_3 = CaHPO_4 + Na_2HPO_4 + 2H_2O + 2CO_2.$$

Urine strongly acid in reaction may contain carbonic acid in solution, but not in combination. Normal urine strongly alkaline in reaction is alkaline from the presence of the carbonates of potassium and sodium. By the process of fermentation ammon. carbonate is formed from the decomposition of urea, hence urine becomes strongly alkaline.

Carbonic acid in the urine, if in any considerable quantity, is detected by acidifying the urine in a test tube with hydrochloric acid, and applying heat, but not to the boiling temperature. If effervescence takes place carbonic acid is present—

$$NaHCO_3 + HCl = NaCl + H_2O + CO_2.$$

CALCIUM AND MAGNESIUM COMPOUNDS.

From the study of the constitution of the phosphates the various combinations of calcium and magnesium, as they are found in the urine, are understood. To test for calcium and magnesium, render the urine distinctly acid with acetic acid, add an excess of a saturated solution of ammon. oxalate, the precipitate formed is calcium oxalate. Filter, and render the filtrate alkaline with ammon. hydrate; the precipitate formed is magnesium ammon. phosphate—

$$\underset{\text{Mag. Ammon. Acet. (double salt).}}{Mg(C_2H_3O_2)_2 2NH_4C_2H_3O_2} + Na_3PO_4 = \underset{\text{Mag. Ammon. Phos.}}{MgNH_4PO_4} + 3NaC_2H_3O_2 + NH_4C_2H_3O_2.$$

The quantity of calcium, calculated as oxide, excreted by the kidneys of an adult in 24 hours is 0.26 to 0.38 grm., and the quantity of magnesium, as oxide, is 0.4 to 0.5 grm. The relative quantity of calcium oxide—the quantity of nitrogen placed at 100—is 0.6 to 1.4, and the relative quantity of magnesium oxide is 0.8

to 1.3. The total quantity of calcium and magnesium oxides excreted in a given length of time is subject to variation, but the relative quantity of each is more constant.

By the ingestion of carbonates, or soluble salts of calcium and magnesium, the total quantity of each in the urine is increased, but the quantity of the salts of each metal absorbed by the blood is estimated at only 10 per cent., while 90 per cent. is excreted with the fæces.

In rickets and osteomalacia, the relative quantity of each is not increased.

AMMONIA.

To test urine for ammonia, to 100 c.c. fresh urine add 200 c.c. alcohol; and a solution of platinum chloride acidified with hydrochloric acid. After standing twenty-four hours filter through a small filter paper, wash with alcohol, dry and transfer to a dry test tube, and heat to redness. The sublimate formed in the test tube is ammon. chloride—

$$Pt(NH_4)_2Cl_6 = PtCl_4 + \underset{\text{Am. Chlor.}}{2NH_4Cl}.$$

By breaking the test tube and placing the sublimate in a small beaker, mixing with some calcium hydrate and moistening with water, ammonia gas will be liberated—

$$2NH_4Cl + Ca(OH)_2 = CaCl_2 + 2H_2O + 2NH_3.$$

Ammonia gas is known by its penetrating odor and alkaline reaction, changing moistened red litmus paper blue, or moistened turmeric paper dark red. The quantity of ammonia excreted by the kidneys of a person of average weight in twenty-four hours is 0.3 to 1.1. The relative quantity—the quantity of nitrogen placed at 100—is 3.5. By the ingestion of any of the mineral acids in medicinal doses, no free acid will appear in the urine, but the quantity of ammonia is increased. Acids in the blood of carnivorous animals increase the quantity of ammonia compounds in the urine at the expense of the quantity of urea. The quantity of ammonia compounds is also increased by a meat diet and diminished by a diet composed principally of vegetables. This fact is accounted for on the theory (Schmiedeberg's) of the formation of ammon. carbonate in the blood from nitrogenous bodies, and by the withdrawal of H_2O from ammonium carbonate

urea is formed, and the ammonia in the urine is a part which has been eliminated from the blood before this action took place. By the ingestion of ammon. carbonate, the quantity of urea excreted is increased, while the amount of ammonia remains about the same, but the quantity of ammonia in the urine is increased by the ingestion of ammon. chloride. These facts are in conformity with the theory. By fermentation of the urine, urea is decomposed, forming ammon. carbonate, and as fermentation of the urine may take place in the bladder, the urine in such cases is usually strongly alkaline when fresh, the alkalinity being due to an increased quantity of ammon. carbonate. To determine this condition of the urine, refer to bacteria, page 21. It sometimes occurs that urine is undergoing the bacterial fermentation, and yet has the acid reaction. This is always the case when the fermentation is started in acid urine, and insufficient time has elapsed to form the amount of ammon. carbonate required to neutralize the acid salts in the urine.

POTASSIUM AND SODIUM.

Evaporate 50 c.c. urine to dryness in a platinum dish, heat the residue over the free flame until the ash appears gray in color. When cool, triturate with some water; filter, and into the filtrate dip a piece of platinum wire which has been heated to redness some time. Now place the end of the wire in the colorless flame (Bunsen's), when the sodium present will impart a yellow color to the flame.

The presence of potassium may be determined by looking at the yellow sodium flame through a piece of glass colored blue by cobalt, when the yellow rays are arrested and a violet color is perceived, due to the presence of potassium. Not having cobalt glass at hand, concentrate the solution (filtrate) to one or two cubic centimetres, and to this add a strong solution of tartaric acid; a precipitate will form—

$$KCl + C_4H_6O_6 = \underset{\text{Acid Potass. Tart.}}{C_4H_5KO_6} + HCl.$$

After standing several hours, filter, wash, dry, and employ the color test, as above, by moistening the end of the wire with some dilute hydrochloric acid, and dipping into the dry powder. In normal urine there is more sodium than potassium. The total

quantity of sodium excreted by the kidneys of an adult in twenty-four hours is 4 to 5 grms. and of potassium 2 to 3 grms. The relative quantity of sodium—nitrogen 100— is 35 to 40, and of potassium 25. Potassium is a constituent of many of the tissues of the body, while sodium compounds are held in solution by the fluids of the body. It was found by Zuelzer that for 100 parts nitrogen in the brain there are 21 parts potassium and 8.7 parts sodium, and for the same number of parts nitrogen in muscular tissue there are 6.1 parts potassium and 1.1 part sodium. As a result of there being more potassium than sodium in the tissue, when there is rapid désassimilation, as in fevers, the quantity of potassium excreted by the kidneys exceeds that of sodium.

CHAPTER III.

Bodies in Diseased Urine—Albuminous Bodies, including Mucine—Serum Albumen—Globuline—Hemialbumose—Peptone—Mucine—Color Tests for Albuminous Bodies—Millon's Reagent—Table of Reactions of Albuminous Bodies, including Mucine—Table of Color Tests—Methods for Determining the Presence of Albuminous Bodies—The Nitric Acid Test—The Acetic Acid and Sodium Chloride Test—Determination of the Presence of Hemialbumose, Peptone, Globuline, and Mucine in Albuminous Urine—Diabetic Sugar—Tests for Sugar in Urine—Trommer's Test Modified by Salkowsky—Test with Fehling's Solution—The Fermentation Test—The Phenylhydrazin Test—Methods for Separating Small Quantities of Sugar from Urine—Inosit—Test for Inosit.

ALBUMINOUS BODIES.

The urine may be regarded as a filtrate of the blood, and by certain diseases of the kidneys or changes in the constitution of the blood or variation in pressure of the blood in the capillaries of the kidneys, the various albuminous substances of the blood plasma appear in the urine; and while in the majority of cases it is sufficient for the physician to know that "albumen" is present, yet in some cases it is doubtless important to determine the presence of other members of this group of bodies. What is generally recognized as albumen in the urine is a mixture of serum albumen and globuline, as these bodies are precipitated by the reagents usually employed in testing for albumen. The quantity of albumen excreted in twenty-four hours differs greatly; 7 grms. may be considered the average quantity, although as much as 30 grms. are sometimes excreted. The principal albuminous body found in the urine is serum albumen; the other members of the class are found in small quantities, and in many cases are absent.

SERUM ALBUMEN.

1. Alcohol precipitates albumen from aqueous solutions, hence, by the addition of alcohol to albuminous urine, the albumen separates in flake-like bodies.

2. Nitric acid precipitates albumen from the urine when added in considerable quantity; in great excess, however, it redissolves. To one volume albuminous urine add six vols. water, when small quantities of nitric acid are added, shaking after each addition.

Albumen will precipitate and redissolve until the acid is added in certain quantity, when the albumen becomes insoluble; therefore nitric acid in small quantities does not precipitate albumen. The object of adding water to the urine is to dilute the solution of neutral salts of the urine, as they aid small quantities of nitric acid in precipitating albumen unless in dilute solutions.

3. Albumen in the urine precipitates by boiling, if the urine is strongly acid in reaction, but by the addition of a solution of sodium hydrate or carbonate, the urine will remain clear by boiling. By heating urine, neutral or slightly acid in reaction, basic phosphates of the metals of the alkaline earths precipitate (refer to phosphates in the urine).

4. Acetic acid does not precipitate albumen in the urine, but when a strong solution of common salt, sodium or magnesium sulphate is added to the urine, acidified with acetic acid, and the urine is boiled, the albumen will precipitate; but without the addition of a solution of one of these salts, an excess of acetic acid is avoided or some of the albumen may remain in solution.

5. By rendering albuminous urine strongly acid with acetic acid, and adding a few drops of a solution of potassium ferrocyanide, albumen will precipitate without the application of heat.

GLOBULINE AND HEMIALBUMOSE.

These bodies are similar in many of their properties. Both are insoluble in water and alcohol, but in water holding in solution neutral salts, alkalies or acid salts, as the urine, they are soluble. Globuline separates from the urine by heating to the boiling temperature with a moderate excess of nitric acid, while hemialbumose remains in solution, but separates as the urine cools. Hemialbumose remains in solution when the urine is treated with ⅓ its volume of a saturated solution of common salt, and acidified with acetic acid, boiled, and filtered while hot. By this process serum albumen, globuline, and mucine are separated from solution.

PEPTONE.

Peptone is soluble in water. Peptone, serum albumen, and hemialbumose are precipitated from solution in the urine by tannic acid, corrosive sublimate and phosphortungstate of sodium with hydrochloric acid. Peptone is not precipitated from solution in

the urine by nitric acid or acetic acid with a strong solution of common salt or acetic acid and potassium ferrocyanide.

MUCINE.

Although mucine is a constituent of normal urine, yet it is often found in greatly increased quantities from cystitis. It is sometimes in shreds or flakes, usually, however, it is in solution. Mucine is precipitated from solution in the urine by adding two volumes of alcohol. It is also precipitated by a solution of tannic acid, acetic acid or a solution of neutral lead acetate. It is quite soluble in water rendered alkaline with potassium or sodium carbonate.

COLOR TESTS FOR ALBUMINOUS BODIES.

Wash some coagulated albumen, saturated with a dilute solution of copper sulphate, on a filter, then transfer some of the coagula to a test tube and treat with a solution of sodium hydrate. The solution becomes deep blue in color.

A solution of peptone rendered alkaline with sodium hydrate, and treated with a small quantity of a solution of copper sulphate, yields a violet color. A solution of hemialbumose, with these reagents, yields a purple violet color.

MILLON'S REAGENT.

For the preparation of Millon's reagent refer to Chapter VIII. To four or six volumes of water in a test tube, containing a small coagula of albumen, one volume of Millon's reagent is added and the mixture heated to the boiling temperature. Particles of albumen will float on the surface of the fluid, if there is an excess of the reagent; if not, they will settle; in either case they are colored light red. Reactions of albuminous bodies with reagents, generally employed for their detection in the urine, are shown by the following table:—

REACTIONS OF ALBUMINOUS BODIES IN THE URINE.

REAGENT.	SERUM ALBUMEN.	GLOBULINE.	HEMIALBUMOSE.	PEPTONE.	MUCINE.
To the urine add nitric acid in excess; the urine is then heated to the boiling temperature.	ppt.	ppt.	ppt. when cold, if the acid is not in great excess, and redissolves when heated, imparting a yellow color.	.....	ppt., and by continued boiling decomposes.
To 5 vols. urine add 1 vol. saturated solution sodium chloride, render strongly acid with acetic acid and heat to the boiling temperature.	ppt.	ppt.	No ppt., but when the urine becomes cold, a ppt. forms. If the urine be saturated with sodium chloride, the hemialbumose will precipitate and not redissolve when the urine is heated.	.....	ppt.
To 1 vol. urine add 3 vols. alcohol, 90 per cent., when the urine is rendered acid with acetic acid.	ppt.	ppt.	ppt.	ppt., partly.	ppt.
To the urine add a sol. of phosphortungstate of sodium, acidified with acetic acid, or a solution of phosphortungstic acid.	ppt.	ppt.	ppt.	ppt.	ppt., if the urine is decidedly acid with acetic acid, otherwise no ppt. forms.
To the urine add acetic acid in excess, when a few drops of a solution of potassium ferrocyanide is added.	ppt.	ppt.	ppt., but redissolves if the urine is heated to near the boiling temperature.	.....	ppt. by the acetic acid.
To the urine add a strong solution of tannic acid.	ppt	ppt.	ppt.	ppt.	ppt., partly from urine, if neutral. The ppt is increased in quantity by adding a solution of sodium chloride.
To the urine add acetic acid in excess without the application of heat.	.....	.....	.....	.....	ppt.

COLOR TESTS.

ALBUMINOUS BODIES, HAVING BEEN SEPARATED FROM THE URINE.

REAGENT.	SERUM ALBUMEN.	GLOBULINE.	HEMIALBUMOSE.	PEPTONE.	MUCINE.
Having saturated the coagulæ with a solution of copper sulphate, introduce into a test tube, and boil with an excess of a solution of sodium hydrate.	blue color.	blue color.	purple violet or blue with more copper sulphate.	purple or violet color.	blue color.
Millon's Reagent—employ as above, page 44.	red color.	red color.	red color.	red color.	red color.

METHODS FOR DETERMINING THE PRESENCE OF ALBUMINOUS BODIES IN THE URINE.

If the urine is turbid, filter, and test the filtrate. In no case should cloudy or turbid urine be employed in testing for albumen. In case the filtrate is turbid, treat the urine with a solution of magnesium sulphate, add a small quantity of a solution of sodium carbonate, shake well and filter; the filtrate will be clear.

1. THE NITRIC ACID TEST.

To the urine in a test tube add ⅓ its volume of nitric acid, specific gravity 1.2; warm a short time, but not to the boiling point. If a flocculent precipitate forms, it is albumen (serum albumen and globuline). By transmitted light the fluid in the test tube will appear clear.

The conditions to be observed in the employment of this test are: The urine should be clear, the acid of certain strength (sp. gr. 1.2), added in definite quantity, and the urine should not be boiled.

2. ACETIC ACID AND SODIUM CHLORIDE TEST.

Render the urine strongly acid with acetic acid, and to five volumes urine add one volume of a saturated solution of common salt,* and heat to the boiling point. If a precipitate forms, it is

* The saturated solution of common salt is prepared by dissolving 350 grms. sodium chloride in one litre water; filter the solution, if it is not clear.

composed of serum albumen and globuline while the solution is hot, but as it cools, hemialbumose also separates. The conditions for the employment of this test are: The solution of sodium chloride should be saturated and added in definite quantity, and the urine should be strongly acid with acetic acid.

METHOD FOR DETERMINING THE PRESENCE OF HEMIALBUMOSE IN ALBUMINOUS URINE.

To five volumes urine add one volume saturated solution of common salt, and render strongly acid with acetic acid; heat to the boiling point and filter while hot. If hemialbumose is present, the filtrate will become cloudy as it cools. To prove that the precipitate so formed is hemialbumose, to the filtrate add an equal volume of the saturated solution of sodium chloride. Hemialbumose will precipitate. Filter, and dissolve the precipitate in strong nitric acid; warm gently; the acid solution becomes deep yellow in color if the body is hemialbumose.

METHOD FOR DETERMINING THE PRESENCE OF PEPTONE IN ALBUMINOUS URINE.

To separate albuminous bodies, except peptone, from the urine, 500 c.c. urine (Hofmeister) is treated with 10 c.c. concentrated solution of sodium acetate, when a solution of ferric chloride is added until, after mixing, it assumes a red color. Boil the solution and filter while hot. The filtrate should yield no precipitate with acetic acid and potassium ferrocyanide. Peptone is separated from the filtrate by adding an excess of a solution of tannic acid, and, after standing twenty-four hours, filtering. Wash the precipitate with water containing some tannic acid and magnesium sulphate. To separate the tannic acid from the peptone, the precipitate is triturated with a saturated solution of barium hydrate in a small mortar, while some crystallized barium hydrate is added. The mixture is then introduced into a small beaker or dish, and heated on a sand bath to the boiling point. After standing a few minutes, filter, and test the filtrate for peptone, employing the color tests as above.

METHOD FOR DETERMINING THE PRESENCE OF GLOBULINE AND SERUM ALBUMEN IN ALBUMINOUS URINE.

Filter the urine if necessary, and if the filtrate is acid or alkaline, render it neutral by employing a solution of sodium car-

bonate or dilute acetic acid. Shake or stir the urine with pulverized magnesium sulphate until it ceases to dissolve. Globuline will separate as a precipitate. Filter, and wash the precipitate with a saturated aqueous solution of magnesium sulphate until the wash water ceases to form a precipitate when acidified with acetic acid and heated to the boiling point. Transfer the precipitate to a beaker and add water until it dissolves. Heat the solution to the boiling point, when, if globuline is present, it will precipitate. The filtrate, after treating with magnesium sulphate, contains the serum albumen, to precipitate which render the solution strongly acid with acetic acid and heat to the boiling point. In the separation of globuline from albuminous urine, instead of employing magnesium sulphate, ammon. sulphate may be employed (Pohl's method). For this purpose render the urine slightly alkaline with ammon. hydrate, and, having stood several hours, filter, to separate the phosphates of calcium and magnesium. To one volume of the filtrate add one volume of a saturated solution of ammon. sulphate. If a precipitate forms, it is globuline.

METHOD FOR DETERMINING THE PRESENCE OF MUCINE IN ALBUMINOUS URINE.

To one volume of urine add three volumes strong alcohol, stir, and let stand several hours. Mucine and all albuminous bodies precipitate. Filter and wash the precipitate with alcohol. Treat the precipitate on the filter with warm water. The filtrate contains the mucine, to precipitate which render strongly acid with acetic acid; if the solution becomes turbid, mucine is present. Refer to reactions of albuminous bodies, including mucine, page 45. In urine not containing albuminous bodies, the presence of mucine is determined by diluting one volume of urine with one volume water and adding an excess of acetic acid; mucine precipitates, causing the urine to appear cloudy or opaque. The object of diluting the urine is to overcome the tendency of the neutral salts of the urine to dissolve the mucine in the presence of an excess of acetic acid.

DIABETIC SUGAR.

Glucose, grape sugar, corn sugar, and starch sugar are synonymous with diabetic sugar. Diabetic sugar is a crystalline body containing water of crystallization. The formula is—

$$C_6H_{12}O_6, H_2O.$$

By drying in a desiccator over concentrated sulphuric acid, it loses its water of crystallization when it melts at 146°. Diabetic sugar is soluble in water, somewhat soluble in alcohol and insoluble in ether. It is not easily decomposed by acids, but decomposes when heated in solution with potassium or sodium hydrate. With potassium hydrate, sodium chloride, or lead acetate with ammon. hydrate, it separates as an insoluble body.

Diabetic sugar has the chemical property of an aldehyde, hence it is easily oxidized to form an acid.

$$C_6H_{12}O_6 + O_2 = H_2O + C_6H_{10}O_7.$$

It is on this property that depends one of the most important tests for it in the urine. By the action of the yeast ferment, it decomposes into alcohol and carbonic acid—

$$C_6H_{12}O_6 = 2C_2H_6O + 2CO_2.$$

Diabetes Mellitus is a disease characterized by sugar in the urine; but it is more than this; one of its prominent features is, that there is rapid désassimilation or waste of the tissues in acute cases, hence there is more urea excreted in a given length of time than could be formed without loss in weight, taking into account the quantity of nitrogenous food consumed. With a meat or nitrogenous diet a greater amount of sugar appears in the urine than could be formed from the starchy or saccharine elements of the food; consequently, in the reduction of albuminous compounds in the blood, sugar is probably one of the products. There are increased quantities of phosphoric and sulphuric acids excreted by the kidneys in diabetes, and the quantity of water drained from the blood is likewise increased, often amounting to four times the normal quantity. Diabetic urine is generally light colored, high specific gravity, caused by the sugar it contains, and acid in reaction.

TESTS FOR SUGAR IN THE URINE.

TROMMER'S TEST MODIFIED BY SALKOWSKY.

For the employment of Salkowsky's modified form of Trommer's test, solutions of sodium hydrate and copper sulphate of known strength are used.

100 grms. sodium hydrate are dissolved in 300 cc. water, and after standing a week, if a sediment forms, decant the solution from it. This solution should be kept in a glass-stoppered bottle, but care should be taken that the stopper and ground surface of the neck of the bottle be free of the alkaline solution, or difficulty in removing the stopper may be encountered. Dissolve 30 grms. crystallized copper sulphate in 300 cc. water. To three volumes of urine add one volume of the solution of sodium hydrate, and of the mixture fill a test tube one-half full and add the solution of copper sulphate, drop by drop, shaking the fluid after each addition, until the copper hydrate ceases to dissolve. Heat the mixture to near the boiling point. If sugar is present, yellow or red cuprous hydrate or oxide will separate. The reduction usually takes place first in the upper stratum of the fluid. The mixture is not boiled, as a few degrees below the boiling point are sufficient for the reduction to take place. There is no difficulty of determining sugar in the urine in the majority of cases, especially if the sugar is in considerable quantity, but as uric acid, coloring matters, kreatinin and other bodies the constitution of which is not known, reduce copper oxide to a certain extent, it sometimes becomes a question, in the employment of this test, if sugar is in the urine.

FEHLING'S SOLUTION.

Qualitative tests for sugar in the urine are made with Fehling's solution. This solution is prepared by introducing 17.32 grms. crystallized copper sulphate in a 250 cc. graduated flask, when water is added, and, the salt having dissolved, the flask is filled with water to the mark and the solution is well mixed by shaking. Introduce into a 250 cc graduated flask 86.5 grms. potassium sodium tartrate and 25 grms. sodium hydrate; add water, and when solution has taken place fill with water to the mark, and finally mix well by shaking. An equal volume of each solution, well mixed, is Fehling's solution. In preparing the solutions for qualitative tests, the weights and measurements may be approxi-

mative. It is not practical to keep Fehling's solution on hand a great length of time, as it is liable to decompose.

Into a small beaker introduce about 10 cc. Fehling's solution and 50 cc. water, heat on a sand bath to near the boiling temperature, when 10 cc. urine is introduced from a pipette, the temperature of the solution still kept near the boiling point a few moments. If the color of the solution becomes green, continue the addition of urine in quantities of 10 cc. until 40 cc. is added. The beaker is then placed in some cold water, so that in case cuprous oxide is formed it may settle rapidly. When cold the solution is decanted, and if cuprous oxide has formed it may be seen on the bottom and sides of the beaker, or, if the cuprous oxide is in small quantity, by surrounding the end of a stirring rod with some filter paper and wiping the surfaces of the beaker, the red oxide is seen on the paper. If the urine contains a decided quantity of sugar, reduction of copper oxide takes place at once, and the solution becomes brick-dust red in color from the presence of cuprous oxide. In the employment of this test, Fehling's solution diluted with water, as above, should be heated in a beaker to near the boiling point for a short time, and, when cold, examined, to see if any reduction of copper oxide has taken place. Without making this preliminary test, mistakes are liable to occur. Occasionally urine, when heated with Fehling's solution, diluted as above, will yield a light yellow precipitate, cuprous hydrate, from the presence of reducible substances other than sugar, but when this takes place the precipitate usually forms while the solution is cooling. In cases of doubt, the urine is decolorized by one of the following methods :—

1. To the urine add a saturated solution of neutral and basic lead acetate until, after stirring and settling, a precipitate ceases to form. An excess of the lead salts is avoided. Filter; the filtrate will be nearly colorless.

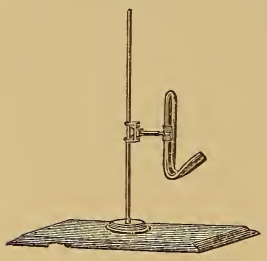
2. A saturated solution of mercuric chloride—corrosive sublimate—is added to the urine until a precipitate ceases to form. The mixture is then rendered alkaline with a solution of sodium carbonate, when it is filtered. The filtrate obtained by the employment of either process, 1 or 2, is examined for sugar by Salkowsky's form of Trommer's test or by the employment of Fehling's solution.

Coloring matters of the urine are removed by boiling the urine with pulverized animal charcoal, but either of the methods described above is preferable, as sugar is absorbed by the charcoal.

THE FERMENTATION TEST.

For this test a piece of combustion tube about 20 cm. long is sealed at one end, and near the other end it is drawn out somewhat and bent, as shown in Fig. 2. The capacity of the tube to the part bent is about 20 cc. Before mixing the urine with the yeast, boil it several minutes so as to drive off CO_2 and other gases. If the urine is alkaline, it is rendered distinctly acid with a solution of tartaric acid before boiling. Pressed yeast is mixed with a small quantity of water, filtered and washed with water on the filter several times, that saccharine bodies which might be present may be removed. About 30 cc. of the urine, having been boiled and cooled, is introduced into a small mortar and triturated or mixed with about 0.5 grm. of the washed yeast by means of a stirring rod. If the mixture is but faintly acid in reaction, it is rendered distinctly acid with a solution of tartaric acid, carefully avoiding the addition of more than is necessary. The tube is then filled with the mixture and placed in position, when a small quantity of mercury is introduced into the open end of the tube to prevent communication between the urine in the different parts of the tube. The apparatus is kept between 15° and 25° C. for twenty-four hours. If sugar is present, CO_2 gas will collect in the sealed extremity of the tube within six hours. In the employment of this test it is necessary to determine the relative quantity of gas evolved from normal urine. For this purpose a test is made by triturating about the same quantity of washed yeast with normal urine, having been acidified, if necessary, boiled and subjected to the same temperature as that of the urine supposed to contain sugar. Normal urine, by the yeast fermentation, yields a small quantity of gas, but the amount is greatly diminished by boiling the urine. If the suspected urine yields a greater volume of gas, sugar is present. If no gas is evolved in either, the yeast

Fig. 2.



may not be effective; it is then triturated with some normal urine, acidified, if necessary, with tartaric acid, and to the mixture a small quantity of cane sugar is added, and the mixture is then introduced into the tube and subjected to a favorable temperature, 20° C.

PHENYLHYDRAZIN TEST.

The employment of this reagent for the detection of sugar in urine has been used to a very limited extent, as its use for this purpose is of recent discovery. 50 cc. urine, supposed to contain sugar, is introduced into a beaker, when 2 grms. phenylhydrazin hydrochlorate with 1.5 grm. sodium acetate, or 1 grm. of the latter if the urine is not decidedly acid, is added. Unless the urine is nearly colorless, add 20 cc. water. The beaker is then placed in a water bath and warmed gently one hour. If sugar is present crystals of phenylglucosazon will form. It was at first supposed that the form of crystals of this body, as found by the microscope, would be sufficient for determining the body; but it was subsequently found that the only positive evidence that the body is phenylglucosazon is obtained by separating it from the urine by filtering, washing with a small quantity of water and dissolving in a small quantity of dilute alcohol, the body crystallizes out by evaporating at a low temperature.

This process is repeated two or three times, when the crystals are collected, dried in a desiccator over concentrated sulphuric acid and the temperature at which the body melts determined. Phenylglucosazon melts at 204 to 205° C. For the purpose of determining the melting point, draw out a piece of thin glass tubing in a Bunsen's flame or a spirit lamp, so that the sealed capillary extremity is 2 or 3 cm. from where the tube is of original diameter. The tube is broken, by means of a file, near where the contraction begins, and a small quantity of the dry body is introduced into the sealed extremity. The piece of tubing is now attached to a thermometer by means of a small rubber band (obtained by making a section of a rubber tube). The capillary end of the tube containing the body is placed adjacent to the bulb of the thermometer, and the bulb with the tube is introduced into concentrated sulphuric acid in a beaker, when the acid is heated gradually. As the mercury ascends to 204° the substance will begin to show evidence of fusion, providing the increase in tem-

perature is gradual and the heat be equally diffused by stirring the sulphuric acid with a glass rod.

METHODS FOR THE SEPARATION OF SUGAR FROM THE URINE.

In case urine contains sugar in small quantity so that the results of the tests for it are not satisfactory, it may be separated from the urine by either of the following methods, and the aqueous solution tested for sugar:—

1. About one litre urine, if not acid in reaction, is rendered acid with hydrochloric acid and evaporated on a water bath to near dryness, when the residue is treated two or three times with small quantities of strong alcohol, and the alcohol solution is filtered. To the filtrate add an alcohol solution of potassium hydrate, stir and let stand two hours. If sugar is present a precipitate will form—

$$(C_6H_{12}O_6)_2K_2O,$$

which is separated from the alcohol solution by decantation and washed once with strong alcohol. Dissolve the precipitate with water, and neutralize the solution with dilute acetic acid; treat the neutral solution with a solution of lead acetate until it ceases to form a precipitate, when it is filtered and the lead is separated from the filtrate with sulphuretted hydrogen gas, H_2S. The solution is filtered from the PbS and the filtrate is concentrated by evaporation on a water bath. During the evaporation the H_2S will escape. Instead of separating the lead by means of H_2S, a solution of pure oxalic acid may be employed, and the excess of oxalic acid removed from the filtrate by mixing well with pure amorphic calcium carbonate. Filter; concentrate the filtrate by evaporating and test the concentrated fluid by any one of the tests above given.

BRUECKE'S METHOD.

To one litre fresh urine add a solution of lead acetate until a precipitate ceases to form; filter, and treat the filtrate with one or two grms. finely powdered basic lead acetate, and after mixing well with a stirring rod, add ammon. hydrate in quantity that after stirring well the odor of the gas is perceptible. The sugar combines with the lead hydrate, as it is precipitated with ammon. hydrate. Filter, and wash once with water, and transfer from the filter to a beaker by means of a fine stream of water from a wash

bottle. The ammonia gas is driven off by heating to near the boiling point, until the vapors given off are no longer alkaline in reaction, when sulphuretted hydrogen gas is passed through the mixture, sugar is set free and PbS formed. Filter, and concentrate the filtrate by evaporating on a water bath. The fluid so concentrated, and free of H_2S gas, is tested for sugar by any of the tests given above. In the employment of this method, the part of the process requiring special attention is the complete separation of ammonia by heating the mixture; for, if some remains in solution, ammon. sulphide will form when the solution is treated with H_2S, which would form CuS with CuO, if either Trommer's test or Fehling's solution be employed for the detection of sugar.

Albumen in urine is first removed before the urine is tested for sugar. For this purpose a solution of tannic acid may be employed. Having precipitated the albumen, it is separated by filtering and the filtrate is tested for sugar. When possible, fresh urine is employed for the detection of sugar.

INOSIT.

Inosit is isomeric with diabetic sugar. It is a crystalline body, soluble in water, but insoluble in absolute alcohol and ether. It differs from diabetic sugar in forming an insoluble compound in neutral solutions with basic lead acetate; and with yeast it does not change into alcohol and carbonic acid, neither does it reduce copper oxide.

SCHERER'S TEST FOR INOSIT.

When an aqueous solution of inosit and calcium chloride is evaporated to dryness and the residue treated with ammon. hydrate, a rose-red color is produced. Inosit sometimes appears in albuminous and sometimes in diabetic urine. Inosituria is a disease dependent on other diseases, although in some cases diabetes mellitus changes into inosituria, that is, sugar disappears from the urine and in its place inosit appears.

TEST FOR INOSIT IN URINE.

One litre of urine is rendered slightly acid, if not already so, with hydrochloric acid and treated with a solution of neutral lead acetate, until a precipitate ceases to form, avoiding the addition

of more than is necessary. Filter, and concentrate the filtrate to one-fourth its volume by evaporating on a water bath, when the solution is treated, while still warm, with basic lead acetate, until a precipitate ceases to form. After standing twenty-four hours, filter, transfer the precipitate from the filter to a beaker by means of a fine stream of water from a wash bottle, and through the mixture pass sulphuretted hydrogen gas. Separate the PbS by filtering after standing a few hours; if uric acid has separated, decant the fluid into an evaporating dish, evaporate to the consistence of syrup and triturate with absolute alcohol. The residue or precipitate, having been washed with absolute alcohol, is dissolved in hot water and tested for inosit, employing Scherer's test. To separate inosit from aqueous solution, add four volumes strong alcohol and sufficient ether to produce a cloudiness, and by standing inosit will crystallize.

CHAPTER IV.

Bodies in Diseased Urine, Continued—Biliary Acids—Tests for Biliary Acids in Urine—Biliary Coloring Matters—Tests for Biliary Coloring Matters—Coloring Matters of the Blood—The Spectroscope—Spectroscopic Test for Hæmoglobin, Oxyhæmoglobin and Methæmoglobin—Heller's and Struve's Tests for Coloring Matters of the Blood in the Urine—The Elements of Blood in Urine—Blood Corpuscles—Separation of Fibrin from Urine—Tests for Fibrin—Leucin—Separation of Leucin from Urine—Tyrosin—Separation of Tyrosin from Urine—Fat—Tests for Fat in Urine—Separation of Fat from Urine—Dreschel's Apparatus—Cholesterin and Lecithin—Method for Separating Cholesterin from Urine—Tests for Neurin and Glycerin-Phosphoric Acid Produced from the Action of Barium Hydrate on Lecithin.

BILIARY ACIDS.

By certain pathological processes in the liver, the bile is retained and absorbed or the liver fails to eliminate the constituents of bile absorbed from the intestines, when the biliary acids and coloring matter with its oxidation products appear in the urine. That the biliary acids are ever found in urine was long a question, until reactions of them became known, by which their presence is determined. Biliary acids combined with sodium differ in constitution in the bile of different animals. In the bile of the ox there are two acids, glycocholic ($C_{26}H_{43}NO_6$) and taurocholic ($C_{26}H_{45}NSO_7$) acids. Each of these acids absorbs water and yields as product of decomposition cholic acid, $C_{24}H_{40}O_5$. Besides this body, glycocholic acid yields glycocol, $C_2H_5NO_2$, and taurocholic acid, taurin, $C_2H_7NSO_3$. The composition and physical properties of the acids in human bile are unknown, but the acid product corresponding to cholic acid is anthropocholic acid, $C_{18}H_{23}O_4$, and in properties it is similar to cholic acid, as it is formed by boiling a solution of human bile with barium hydrate. It is monobasic, crystalline, and insoluble in water, but soluble in alcohol and ether. The acids in human bile corresponding to glycocholic and taurocholic acids have not been separated.

PETTENKOFFER'S TEST FOR BILIARY ACIDS.

To three parts of a solution of biliary acid compounds add two parts of strong sulphuric acid gradually, so that the temperature remains under 60° C., then add three drops of a solution of

cane sugar (1 part cane sugar and 4 parts water) and shake well. The solution becomes violet in color.

TEST FOR BILIARY ACIDS IN URINE.

On account of the presence of indican in the urine, the presence of biliary acids in icteric urine cannot always be determined without first separating them from the urine. As the biliary acids are never found in the urine in great quantity, one litre of the urine (Neukomm) is evaporated on a water bath to near dryness, the residue triturated with strong alcohol, filtered and the process repeated several times. The alcohol filtrate is evaporated to dryness on a water bath, treated with absolute alcohol several times, and the alcohol solution is filtered. The biliary acids are thus separated from bodies in the urine insoluble in alcohol. Evaporate the alcohol filtrate on a water bath to dryness, and treat the residue with water, and after stirring several minutes add finely pulverized basic lead acetate until a precipitate ceases to form, when a few drops of a solution of the salt is added. After standing several hours the solution is filtered and the precipitate washed with some water. Transfer the precipitate to a flask, treat with alcohol, 85 per cent., boil on a water bath a short time, and filter while hot. The lead compounds of the biliary acids are insoluble in water but soluble in alcohol while hot. To the filtrate add a solution of sodium carbonate to alkaline reaction and evaporate on a water bath to dryness. Boil the residue with absolute alcohol, filter while hot into a flask, and, having become cold, add ether until a precipitate forms, close the flask with a stopper, and let stand twenty-four hours. Sodium salts of the biliary acids will crystallize. Decant the fluid and dissolve the crystals in some water, and test the solution with Pettenkoffer's reagents.

COLORING MATTERS OF THE BILE IN URINE.

Bilirubin, $C_{16}H_{18}N_2O_3$, is the principal coloring matter of the bile. Of this, however, there are several oxidation products, the principal one being biliverdin ($C_{16}H_{20}N_2O_5$, according to Stædeler). Bilirubin is a solid, insoluble in water but soluble in chloroform. Solutions of alkaline carbonates or hydrates dissolve it. With calcium salts it forms insoluble bodies in water. Bilirubin, when dissolved in an aqueous solution of sodium hydrate or carbonate

and exposed to the air, oxidizes to form biliverdin. Biliverdin is a green solid, insoluble in water and chloroform, but soluble in alcohol, and like bilirubin, it dissolves in solutions of the alkaline carbonates or hydrates, and precipitates from solution with soluble salts of calcium. Bilirubin, absorbed by the blood from the intestines, is separated from the blood by the liver, and not by the kidneys, unless the quantity in the blood is greatly increased. Pathologists have found that bilirubin is also formed in the blood by processes which diminish the number of red corpuscles, and that the liver, although not structurally diseased, may fail to take from the blood the amount of bilirubin formed, in which event the kidneys accomplish the work. This form of disease—hæmatogenous icterus—is distinguished by the presence of biliary coloring matters, and absence of biliary acids in the urine while in hepatogenous icterus, both coloring matters and biliary acids are found in the urine.

TESTS FOR BILIARY COLORING MATTERS IN URINE.

Icteric urine may be recognized by its physical properties. The color is dark yellow, sometimes brown, and, when shaken, forms a yellow foam.

GMELIN'S TEST.

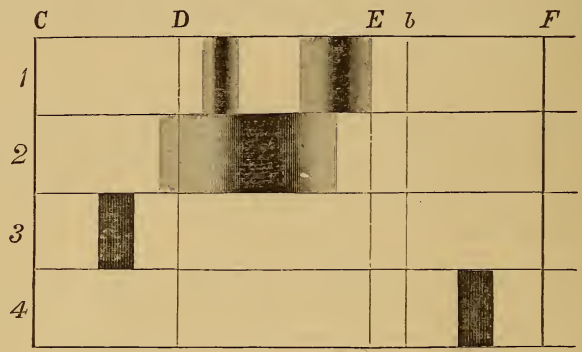
Into a test tube one-fourth full of nitric acid containing some nitrous acid—yellow nitric acid formed by exposure to light—let run down the side of the test tube some of the urine, so that it will remain on the acid. This is effected by holding the test tube containing the acid as near horizontal as possible, while the urine runs in from another test tube. The point of contact of urine and acid will undergo several changes in color, beginning with green and ending with yellow. The green color which appears in the reaction is characteristic of biliary coloring matters. A modification of this test is to filter, dry the filter paper carefully, and by means of a glass rod to place a drop of nitric acid on the paper. A colored ring will soon form, enclosing the place moistened by the acid. In case the coloring matters are in small quantity, introduce about 50 cc. of the urine into a glass-stoppered 100 cc. cylinder, render acid with acetic acid, and shake some time with about 20 cc. chloroform. Draw off the chloroform solution by means of a syphon or pipette, and introduce into a

100 cc. glass cylinder containing 50 cc. water, in which 5 or 10 grms. sodium carbonate are dissolved. After shaking some time, draw off the aqueous solution and test by employing Gmelin's test.

COLORING MATTERS OF THE BLOOD.

Hæmoglobin is the coloring matter of the red corpuscles of the blood. By heat it is decomposed into hæmatin and albumen. A solution of hæmoglobin is dark violet in color. It absorbs oxygen when exposed to the air, forming oxyhæmoglobin, which imparts a deep red color to its solutions. Hæmoglobin and its oxidation product have different optical properties. A solution of hæmoglobin placed in a glass cell with parallel walls (*a*, Fig. 3), and

TABLE I.

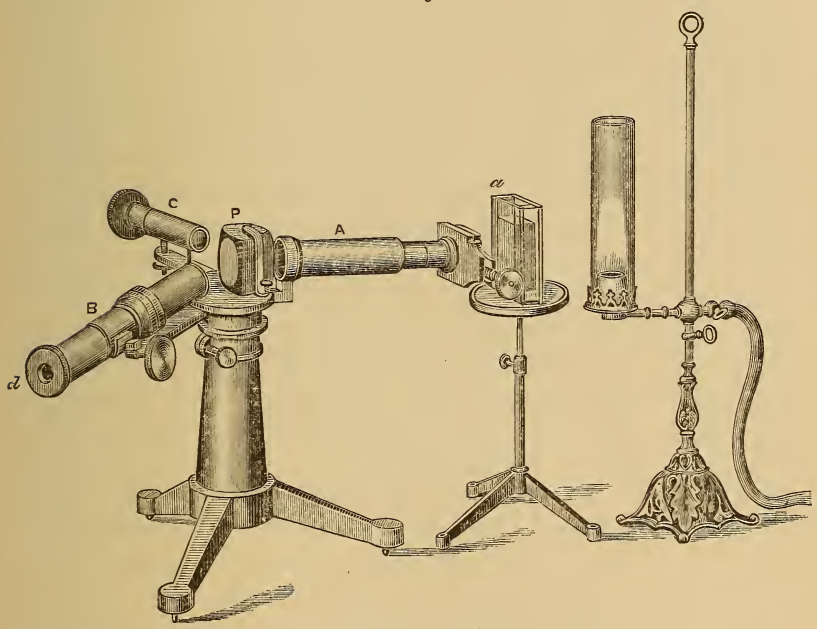


rays of light from a lamp passed through the solution, will reveal, in the spectroscope, a broad band, as shown by Spectrum 2, Table 1, between D and E; but as hæmoglobin absorbs oxygen, the spectrum of the oxygen compound—oxyhæmoglobin—may likewise be seen, which consists of two bands or lines, one yellow and the other green, between D and E, Spectrum 1, Table 1. By reducing oxyhæmoglobin, when in solution, by means of a few drops of ammon. sulphide, and examining the solution with the spectroscope, the broad band, Spectrum 2, will be seen, with the absence of the yellow and green bands, Spectrum 1.

By the action of acid salts on a solution of either hæmoglobin or oxyhæmoglobin, it changes to methæmoglobin, a solution of which, by examination with the spectroscope, yields a well-defined

red line between C and D, Spectrum 3, Table 1. When the coloring matter of the blood is in the urine, this is the usual form in which it is found; but as methæmoglobin in urine, undergoing fermentation, changes to hæmoglobin, and as the latter changes to oxyhæmoglobin by exposure to the air, all three bodies may be found in urine. By heating a neutral or acid solution of either hæmoglobin, oxyhæmoglobin or methæmoglobin, the albumen formed by the decomposition coagulates, and is colored from

FIG. 3.



(*From Neubauer.*)

hæmatin. Decomposition of these bodies likewise takes place by the action of glacial acetic acid and common salt, when warmed in the absence of water. As hæmatin is liberated by heat, it is not found in urine containing the coloring matters of the blood.

TESTS FOR COLORING MATTERS OF THE BLOOD IN URINE.

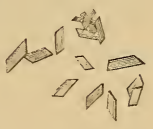
When coloring matter of the blood is in the urine, it is either enclosed in red corpuscles, as in hæmaturia, or in solution in the urine, hæmoglobinuria, produced by the action of certain toxi-

cants, as the mineral acids, potassium chlorate, and the poison of scarlet, and other infectious fevers. The different parts of the spectroscope are adjusted by first removing the prism, P (Fig. 3), and placing the tube, A, that light from the lamp passing through the slit illuminate the margins of the tube, which is determined by looking through it toward the lamp. The telescopic part of the apparatus, B, is adjusted that objects at a distance are distinctly seen. The prism is now placed in position, and the reflecting tube, C, is adjusted that light be reflected through the telescope, and the scale become visible by the observer at *d*. By excluding light from the prism by a black cloth placed over it, and with the admission of a bright light into the reflecting tube, the apparatus is ready for use. A glass cell with parallel walls, *a*, is filled with urine for examination. If the urine is alkaline, render it acid with acetic acid. The urine, if turbid, is filtered, and the filtrate is examined, but if no spectrum indicating the presence of coloring matters of the blood is observed, the urine, before filtration, is examined. Highly-colored urine may be treated with finely-pulverized basic lead acetate until a solution of it ceases to produce a precipitate, and, after mixing well by shaking, render alkaline with ammon. hydrate, filter, and examine the filtrate with the spectroscope. The filtrate contains any oxy-hæmoglobin and hæmoglobin that may be in the urine, and the precipitate contains the methæmoglobin. If the result of the examination of the filtrate is unsatisfactory, wash the precipitate with water, transfer to a beaker by means of a fine stream of water from a wash bottle, and treat the precipitate suspended in water with a solution of sodium carbonate. The lead compound of methæmoglobin is decomposed by sodium carbonate with the formation of lead carbonate and methæmoglobin set free. Filter, and examine the filtrate with the spectroscope. In case the urine contains biliary coloring matters in sufficient quantity to interfere with the spectroscopic examination for the coloring matters of the blood, the former are removed by rendering the urine slightly alkaline with ammon. hydrate, and adding a solution of calcium chloride until, after mixing well by stirring, a precipitate ceases to form, when the filtrate is examined with the spectroscope. The spectra of the coloring matters of the blood are described above.

HELLER'S TEST.

Not having a spectroscope at hand, the chemical tests will answer the purpose. Render the urine strongly alkaline with a solution of sodium hydrate and heat to the boiling point. The precipitate formed is composed of calcium and magnesium phosphates, colored red by hæmatin. This test alone is generally sufficient, but to examine further, filter, wash the precipitate with a small quantity of water and dry at 100° C. Introduce the dried mass into a clean, dry test tube, add a small crystal of common salt and treat with some glacial acetic acid, heat to the boiling temperature, filter a small quantity (1 cc.) through a very small filter into a watch glass, evaporate on a water bath at 50° to 60° C.,* and examine the residue with the microscope. Crystals of hæmatin (Fig. 4) are found if coloring matter of the blood is in the urine. In order to employ the microscopic test, without regard to the red color of the precipitate, with sodium hydrate, the coloring matter is separated (Struve) by rendering the urine alkaline with a solution of sodium hydrate, when a solution of tannic acid is added until a precipitate ceases to form; then render acid with acetic acid, filter, wash with water, dry, and transfer to an agate mortar. The dried mass is now triturated with a small crystal of sodium chloride, when it is transferred to a dry test tube and boiled with some glacial acetic acid. Filter through a small filter into a watch glass and evaporate to dryness at a low temperature. The residue is examined for hæmatin with the microscope.

Fig. 4.



BLOOD IN URINE.

If blood is in the urine besides hæmoglobin or its derivatives, the urine is examined for red corpuscles, albumen and fibrin. Urine containing blood is usually red in color, yet the color

* For this purpose the method of evaporating at a low temperature, first suggested by Streng, may be employed. Place a circular piece of glass over an evaporating dish containing water, the margin of the glass not extending more than 5 mm. over the dish. The water in the dish is heated to the boiling temperature, and a watch glass or slide, in or on which is the solution to be evaporated, is placed on the glass plate. Evaporation will take place slowly. To evaporate at a still lower temperature, introduce a piece of thin paper between the watch glass or slide and the glass plate.

varies from red to dark brown, depending on the quantity of blood present and on the condition of the urine, whether fresh or undergoing fermentation.

To determine the presence of red corpuscles of the blood, refer to Urinary Sediments, Chapter v, and for albumen, refer to page 46. Albumen is always found in small quantity in urine containing hæmoglobin or its derivatives, but if red corpuscles of the blood are present, the quantity of albumen is greater coming from the liquor sanguinis as well as from decomposition of the coloring matters of the blood. If, however, the number of red corpuscles is not great, and the quantity of albumen is considerable, the indication is that the excess of albumen originates from a diseased condition of the kidneys producing albuminuria. If albumen, separated by heating some of the urine acidified with acetic acid to the boiling temperature, comes from the blood proper in the urine, it will rise to the surface in the form of highly-colored flakes. Fibrin usually appears as small coagulæ suspended in the urine. To separate them from the urine, filter through fine muslin and wash with cold water. Treat a part of the mass supposed to be fibrin with a dilute solution of sodium hydrate; if insoluble, the indication is that it is fibrin; albuminous bodies dissolve in sodium hydrate. Treat another portion with a weak solution of sodium carbonate (1 part Na_2CO_3 dissolved in 100 parts water). Fibrin dissolves completely in this solution if warmed gently several hours on a water bath. This solution is then filtered and tested with Millon's reagent, page 44, when a deep red color is produced.

LEUCIN.

Leucin and tyrosin are found in the urine as a result of certain pathological processes, particularly in acute yellow atrophy of the liver and poisoning by phosphorus. The formula of leucin is—

$$C_6H_{13}NO_2.$$

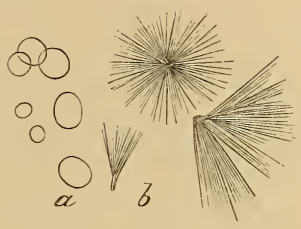
It is an oily-like solid crystallizing in spherical bodies, *a*, Fig. 5. It is partly soluble in water, slightly soluble in alcohol and insoluble in ether. It is not found in urinary deposits.

TEST FOR LEUCIN IN URINE.

One litre of fresh urine is treated with an excess of basic lead acetate. Filter, and precipitate the lead from the filtrate with

sulphuretted hydrogen gas. The lead sulphide is separated from the solution by filtering, when the solution is evaporated to dryness on a water bath, and the residue treated with strong alcohol several times, and the alcohol washings are filtered each time into a beaker. Evaporate the alcohol solution at a low temperature, and examine the crystals which separate from solution during evaporation, when, if leucin is present, the form of crystals as shown by *a*, Fig. 5, will be recognized. Place some of the crystals on a piece of platinum foil and heat gradually (Scherer); if leucin is present, a spherical, oil-like globule will form which does not adhere to the foil.

FIG. 5.



TYROSIN.

The formula of tyrosin is—

$$C_9H_{11}NO_3.$$

In water it is slightly soluble, and in absolute alcohol and ether it is insoluble. In a solution of potassium, sodium or ammonium hydrate or carbonate it dissolves. It is also soluble in dilute acids. Tyrosin crystallizes in needle-like crystals, *b*, Fig. 5. With Millon's reagent (page 44) a hot water solution of tyrosin yields a dark-red color (R. Hoffmann). With an excess of concentrated sulphuric acid gently warmed, a light-red color is produced, which changes to a violet red by the addition of a solution of ferric chloride (Piria). By carefully evaporating a solution of tyrosin with nitric acid in a small porcelain dish, a deep yellow color is produced, which changes to dark red by the addition of sodium hydrate (Scherer).

TEST FOR TYROSIN IN URINE.

Tyrosin is sometimes found as a sediment, refer to page 77. Tyrosin is separated from the urine by the process employed in separating leucin, but being less soluble in strong alcohol than leucin, it remains undissolved by treating with absolute alcohol. The residue containing it is treated with weak alcohol heated to the boiling temperature, and filtered while hot. By evaporating

the filtrate, crystals of tyrosin separate, which are examined by the microscope. They are represented by *b*, Fig. 5. For further proof, employ Hoffmann's and Scherer's tests.

FAT.

In the course of a great many pathological processes fat appears in the urine in some cases in the form of oil drops on the surface of the urine, and again as an emulsion; in either form the urine is turbid and does not become clear by heating. In health, fat sometimes appears in the urine, when there is an excess of it in the blood from the ingestion of fats. Resulting from degenerations of the kidneys, liver and other organs, as brought about by cancer, phosphorus poisoning, gangrene, Bright's disease, yellow atrophy of the liver, fractures of the bones and occasionally from diabetes mellitus, fat appears in the urine. Lipuria is therefore symptomatic of various diseases. In chyluria, fat with albumen, lecithin and cholesterin is in the urine in the emulsified form. The urine is white and is more or less homogeneous. This disease depends on the presence of microscopic organisms in the blood—the Filaria sanguinis.

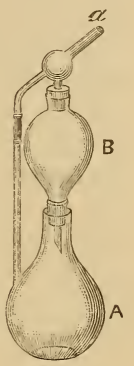
TESTS FOR FAT IN URINE.

The presence of fat in the urine, when enclosed in albuminous casts and epithelial cells, is determined by microscopic examination. Oil drops and globules of fat are represented by Fig. 6. In chyluria the fat is in the emulsified condition, and its presence is determined without difficulty by microscopic examination. If fat is in the form of drops floating on the surface of the urine, its presence may be determined by causing the absorption of the drops by white paper, and by drying the paper the fat is found to be fixed, and the parts of the paper containing the fat are difficult to wet with water. To separate fat from the urine, shake about 400 cc. urine in a 500 cc. glass-stoppered cylinder with 50 cc. ether and decant the ether into a distilling flask. This process may be repeated several times; when the ether is distilled on a water bath, the residue is treated with ether and the ether solution is introduced into a high beaker and evaporated. Fat in the residue is tested by its insolubility in water, by the

Fig. 6.

paper test and by heating in a platinum dish, when a penetrating odor is produced. Instead of shaking the urine with ether, Dreschel's apparatus, Fig. 7, for the extraction of fats, may be employed for this purpose. Evaporate one litre of urine on a water bath to dryness, mix the residue with an insoluble substance, as calcium or barium sulphate, introduce the mixture into a folded filter, which is placed into the flask, B, while the flask, A, is filled about one-half full with ether, and the tube, *a*, is connected with a condenser.

Fig. 7.



The ether is distilled by heating the flask, A, on a water bath, when the vapor will pass up the side tube, and when condensed in the condensing tube, the ether will percolate through the mixture in the filter, dissolving the fat. By continuing the process thirty or forty minutes, the fat will be dissolved. The ether solution is transferred to a high beaker and evaporated to dryness, when the presence of fat in the residue is determined as above.

CHOLESTERIN AND LECITHIN.

Cholesterin, $C_{24}H_{44}O$, is a crystalline body, forming large tabular crystals. It is insoluble in water, soluble in ether and chloroform. When a chloroform solution of cholesterin is treated with an equal volume of strong sulphuric acid, it turns yellow; with an excess of cholesterin the color is red or purple (Salkowsky). Cholesterin undergoes no change when a solution of it is heated with potassium, sodium, ammonium or barium hydrate.

Lecithin, $C_{42}H_{84}NPO_9$, is a solid easily decomposed by heat. It is insoluble in water, soluble in ether and chloroform. When a solution of lecithin is heated with barium hydrate or hydrates of the metals of the alkalies, it decomposes into neurin and glycerin-phosphoric acid.

The method (Hoppe-Seyler and Eggel) employed for determining the presence of cholesterin and lecithin in urine is based on their properties given above. As these bodies are never found in large quantities in urine, they with fat are separated from four or five litres of urine. The urine, in portions of 500 or 1000 cc., is shaken up with ether after having been acidified, if not already

acid in reaction. The ether solution having been drawn off by means of a syphon, the urine is rendered alkaline with a solution of sodium hydrate, and shaken with ether in the same way. The ether solutions of both acid and alkaline urine are distilled on a water bath, the residue treated with ether, free of water, and the solution is transferred to a 400 cc. flask. The ether having been distilled from the 400 cc. flask, the residue is boiled about two hours in 200 cc. of a saturated solution of barium hydrate, with a condenser connected with the flask, placed at about 35 degrees, so that the water condensed will return to the flask. At the end of the process, the fat will be decomposed into glycerin and fatty acids; the latter forming an insoluble soap with barium. Lecithin will be decomposed into neurin and glycerin-phosphoric acid, the latter forming a salt with the barium present, while cholesterin remains unchanged. The residue, cholesterin and barium soap, is separated by filtering, and, having been washed with some water, it is transferred to a 50 cc. glass cylinder, treated with ether in small portions, and after shaking with each portion, the ether solution is decanted from the residue into a high beaker. The residue is finally treated with a mixture of absolute alcohol and ether, and the solution added to the ether solution in the beaker, when, by slow evaporation, cholesterin will crystallize out. Examine the crystals with the microscope; the crystals are tabular in form. Prepare a chloroform solution and test with strong sulphuric acid, as above.

Neurin, one of the products of the decomposition of lecithin, is in solution in the filtrate from the residue of cholesterin and barium soap.

To separate barium hydrate, which is also in the filtrate, pass carbonic acid gas through the solution, and separate the $BaCO_3$ by filtering.

Evaporate the filtrate on a water bath to dryness and treat the residue with absolute alcohol. The neurin will dissolve in alcohol, and the glycerin-phosphate of barium will remain undissolved. Filter through a dry filter paper and treat the filtrate with an alcohol solution of platinum chloride containing some hydrochloric acid. A precipitate forms, composed of platinum chloride and neurin. Filter, and wash with a small quantity of alcohol, and dissolve on the filter with water. The aqueous solution is

concentrated by evaporation at a low temperature, better in vacuo, when the neurin compound will crystallize in six-sided crystals, known by microscopic examination.

The presence of glycerin in the glycerin-phosphate of barium, from which the neurin was dissolved with absolute alcohol, is determined by drying some of it, and heating in a small porcelain dish, when the odor of acrolein will be perceptible. The odor of this body is characterized by its irritating properties. The test for glycerin is made with greater certainty (Hoppe-Seyler) by triturating some of the dried substance with acid potassium sulphate, and heating the mixture in a dry test tube.

CHAPTER V.

Sediments in Urine—Sediments Peculiar to Acid Urine—The Acid Urates and Uric Acid—Hippuric Acid—Calcium Sulphate—Sediments Peculiar to Urine of Strong Alkaline Reaction—Calcium and Magnesium Phosphates and Magnesium Ammon. Phosphate—Ammonium Acid Urate—Calcium Oxalate—Calcium Carbonate—Sediments not Depending on Reaction of the Urine—Cystin—Tests for Cystin in Solution in Urine—Cystin as a Sediment—Tyrosin—Epithelial, Albuminous and Blood Casts—Blood Corpuscles—Pus—Spermatozoa, Bacteria, Sarcina, and Other Microörganisms.

SEDIMENTS PECULIAR TO URINE OF STRONG ACID REACTION.

THE ACID URATES AND URIC ACID.

For the constitution and properties of uric acid and the urates refer to page 22. This sediment is usually red, yellow or "brick dust" in color. By microscopic examination, the acid urates are found to be amorphic or granular, seldom crystalline, except ammonium urate, which is sometimes present. A sediment composed of the urates disappears by heating the urine in which it is suspended and reappears as the urine cools, the acid urates being more soluble in hot than in cold water. Separate the sediment composed of urates from the urine by filtering, wash with a small quantity of cold water, transfer to a test tube, mix with some water and add a solution of sodium hydrate or carbonate; the acid urates will dissolve, owing to the formation of neutral urates, which are quite soluble. For this reason the acid urates may form as a sediment in urine of acid reaction, but not in urine alkaline by the presence of potassium or sodium carbonate. Besides the solubility of the acid urates, by warming the urine containing the sediment, the granular form and the red color of the sediment, the urates may be still further tested by determining if they are soluble in a solution of sodium hydrate with the microscope. For this purpose a small quantity of the sodium hydrate solution is brought on the margin or at one side of the glass cover, when the granular matter dissolves as the alkaline solution comes in contact with it.

Uric acid, as a sediment in fresh urine, is not of such frequent occurrence as in urine having stood some time. By standing, the acid phosphates react on the neutral and acid urates, combining

with some of their bases, and liberate more or less uric acid. For this reason the quantity of free uric acid in the sediment of urine having stood several hours depends on the acidity of the urine and the relative amount of urates present. As this is generally a slow process, the crystals of uric acid are well formed. The forms of the crystals of uric acid as generally found in urinary sediments, and the amorphic or granular character of the urates, are represented by Fig. 8. Uric acid, separated from combination by means of an acid, crystallizes in forms different than when spontaneously separated in the urine. The forms of crystals of uric acid separated by an acid are represented by Fig. 9. It is important, as regards the danger of the formation of concretions in the bladder, to ascertain if in urinary sediments of fresh urine there is uric acid, or if it forms by standing before the urine loses its acidity. To separate uric acid from the urates

FIG. 8.

FIG. 9.

FIG. 10.

in a sediment, heat the urine with sediment, filter while hot, and wash with hot water. The residue is tested with nitric acid and ammon. hydrate, page 23. A heavy deposit of urates and uric acid is no evidence of an increased quantity of uric acid in the urine, for, if there is present no excess of the acid phosphates, the bases may be in sufficient quantity to form neutral urates, and hence uric acid be in great excess without the formation of a sediment containing urates or uric acid; therefore, to determine if uric acid is in excess in urine, quantitative estimations are made.

HIPPURIC ACID.

Hippuric acid is not of frequent occurrence in urinary deposits. The acid crystallizes in rhombic prisms and needle-like forms (Fig. 10), resembling the crystals of magnesium ammon. phosphate. It differs, however, from the latter in appearing as a sediment in urine strongly acid in reaction, and by its insolubility

in acetic acid, which is readily determined in the field of the microscope, with a drop of acetic acid placed at the margin of the glass cover. It sometimes occurs that crystals of hippuric acid, needle-like in form, are attached to crystals of uric acid when the latter are larger in size than are generally found in urinary deposits. Hippuric acid is readily distinguished from uric acid by its solubility in warm water and also in acetic ether.

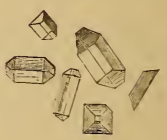
CALCIUM SULPHATE.

The conditions for the formation of calcium sulphate as a sediment are concentration of the urine and strong acid reaction.

FIG. 11.

FIG. 12.

FIG. 13.



There are but a few cases reported of calcium sulphate having been found as a sediment in urine. Its crystals are needle-like and prismatic in form, as represented by Fig. 11.

SEDIMENTS PECULIAR TO URINE OF STRONG ALKALINE REACTION.

CALCIUM AND MAGNESIUM PHOSPHATE AND MAGNESIUM AMMONIUM PHOSPHATE.

Although alkaline urine is generally turbid when fresh, it is sometimes nearly clear, when by standing or gently warming it becomes cloudy, a thin film forming on the surface before a deposit forms. The basic phosphates of calcium and magnesium, as found by the microscope in urinary deposits, are amorphic,

and the granules or particles are sometimes so fine that they are seen with difficulty, unless glasses of high power be employed. The granular character of this sediment is illustrated by Fig. 12. Well-formed crystals of basic magnesium phosphate have been found in urinary deposits, but they are of rare occurrence. They are long, tabular in form, with pointed extremities. On the other hand, crystals of magnesium ammonium phosphates are frequently found. The crystals are usually well formed, large in size, rhombic in form, and resemble, somewhat, a coffin lid (Fig. 13). As there is a comparatively small amount of ammonia salts in normal urine, unless it has undergone fermentation, the quantity of this salt is limited. The phosphates are quite soluble in acetic acid. They do not dissolve by heating the urine in which they are suspended or by the addition of potassium or sodium hydrate. By placing a drop of acetic acid or a solution of sodium hydrate at the margin of the glass cover, under which there is some of the sediment, the action of either of these reagents is determined with the microscope, or, to determine if the sediment is soluble in the urine when warmed, the slide may be carefully warmed, and the effect, if any, noted while the preparation is still warm. The mere fact that in fresh urine these phosphates form a sediment, is of no importance, for they separate from normal urine when alkaline and normal urine becomes alkaline by a vegetable diet. All of the phosphoric acid in alkaline urine is not combined with calcium and magnesium, but more or less remains in solution combined with potassium and sodium. It is important, however, to ascertain if the urine continues alkaline even by varying the diet, and if so, microscopic examination of the urine is made to determine if micrococcus ureæ are present, which is evidence of fermentation of the urine. If the alkaline reaction is due to ammonium carbonate, red litmus paper, when wet with the urine, will turn blue, but when dried the red color will be restored, or, by bringing the end of a glass rod, wet with strong hydrochloric acid, near the surface of the urine, a cloud will form where the vapor of ammonium carbonate comes in contact with the acid.

In urine neutral, slightly alkaline, or acid, the neutral phosphate of calcium—

$$CaHPO_4,$$

sometimes appears as a sediment, the crystals of which are lance or wedge shape, often forming rosettes (Fig. 14).

AMMONIUM ACID URATE.

Ammonium acid urate appears as a sediment, the result of fermentation, when the ammonia formed decomposes the urates of potassium and sodium with the formation of ammonium acid urate. It is nearly insoluble in water. Its crystals are spherical in form, some having fine protuberances resembling the wild gooseberry (Fig. 15). In urine not containing an excess of ammonia, the NH_4 combines by preference with phosphoric acid and magnesium, forming $MgNH_4PO_4$, rather than with uric acid. It is only in urine containing ammonia from fermentation, or urine to which an ammonium compound has been added, that ammonium

Fig. 14.

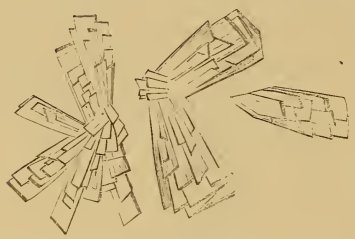


Fig. 15.

Fig. 16.

urate appears in considerable quantity as a sediment. Dilute hydrochloric or acetic acid decomposes ammonium urate with the separation of uric acid. Uric acid separated in this way is represented by Fig. 9, page 71.

CALCIUM OXALATE.

Calcium oxalate crystallizes from neutral or alkaline urine in octahedral crystals, and by the microscope the margins of the crystals, as they cross, are seen, which suggested the idea of the appearance of an envelope (Fig. 16). Occasionally the crystals are dumb-bell or spherical in form. The crystals of calcium oxalate are exceedingly small in size, and when not accompanied by other bodies may escape detection. If the sediment is small in quantity, filter the urine in which the sediment is suspended

through a small Swedish filter paper, wash with some water, transfer to a small beaker by means of a fine stream of water from a wash bottle and a hair pencil, and examine the sediment with a microscope. With crystals of calcium oxalate, calcium, and magnesium phosphates (granular sediment), ammonium urate (spherical crystals), and magnesium ammonium phosphate (rhombic crystals) are usually found. To determine the presence of calcium oxalate in the sediment of urine strongly alkaline in reaction, filter as above and dissolve the phosphates on the filter with dilute acetic acid, and, having washed with water, transfer the residue to a small beaker, as above, and examine for calcium oxalate with the microscope. As calcium oxalate differs from the phosphates in being insoluble in acetic acid, and from the urates (acid) and uric acid, in being insoluble in a solution of sodium hydrate, these reagents are employed to distinguish the oxalate from these sediments. The reactions may be made on the glass slide by placing a drop of the reagent at the margin of the glass cover, and observing what takes place as the reagent passes in the field of view. The quantity of oxalic acid in the urine is not increased by any disease, as far as has been determined, and regarding the existence of oxaluria pathologists do not agree. In nearly all the researches made on this subject, the oxalic acid combined with calcium as a deposit in alkaline urine, as found by microscopic examination, has been taken into account, but no quantitative estimations were made. As the question turns on quantity rather than the presence of oxalic acid in the urine, investigations made so far are of but little value.

CALCIUM CARBONATE.

Calcium carbonate is not often found as a sediment. It generally forms by a continuous vegetable diet, when the urine becomes alkaline. From the urine it generally separates in spheroidal crystals, which occasionally become connected, so as to resemble dumb-bells. The forms of crystals, as generally found in urinary sediments, are represented by Fig. 17. Calcium carbonate dissolves in dilute acetic acid with the liberation of carbonic acid gas. If the quantity of calcium carbonate in a sediment is small, this reaction is made in the field of the microscope by placing a drop of the acid at the margin of the glass cover, and when

it comes in contact with the crystals, gas will evolve, leaving no doubt as to the constitution of the crystals.

SEDIMENTS NOT DEPENDING ON REACTION OF THE URINE.

CYSTIN.

The formula of cystin is $C_3H_7NSO_2$. It is seldom found in urine, and its presence is pathognomonic of no particular disease. It is found mostly in sediments and concretions. Cystin is insoluble in water, ether, alcohol and acetic acid; soluble in hydrochloric acid and a solution of potassium, sodium or ammon. hydrate. When cystin is dissolved in a solution of sodium hydrate, and boiled with some lead hydrate, a black precipitate is formed—lead sulphide; the cystin is decomposed, and the sulphur in it combines with the lead. When some sodium nitro-

FIG. 17.

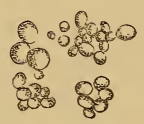


FIG. 18.

prusside is added to a hydrate of sodium solution of cystin, the solution turns violet. Cystin crystallizes in six-sided prisms (Fig. 18).

TESTS FOR CYSTIN DISSOLVED IN THE URINE.

Treat 500 cc. of the urine (Loebisch) with an excess of acetic acid; filter, wash with water and dissolve on the filter with water containing ammon. hydrate. Evaporate the filtrate in a beaker on a water bath, and cystin will separate from solution in crystals. Examine the crystals with the microscope and prepare a hydrate of sodium solution, and test with a small quantity of a solution of sodium nitroprusside, when the solution becomes purple in color, if cystin is present.

CYSTIN AS A SEDIMENT.

Filter the urine containing the sediment, wash with water, and dissolve on the filter with water containing ammon. hydrate.

Render the ammonia solution distinctly acid with acetic acid and separate the cystin which precipitates by filtering, when some of the precipitate is dissolved in a solution of sodium hydrate and tested with a small quantity of sodium nitroprusside. If the solution turns purple or violet in color, cystin is present.

TYROSIN.

Tyrosin, when in a sediment, is separated from other constituents of the deposit by the method employed above in separating cystin. Crystals of tyrosin precipitated by acetic acid are dissolved in dilute ammon. hydrate, to which some ammon. carbonate has been added, filtered, if necessary, and evaporated at a low temperature, when tyrosin will separate in the form of crystals, *b*, Fig. 5, page 65. Examine the crystals with the microscope, and test according to R. Hoffmann and Scherer, page 65.

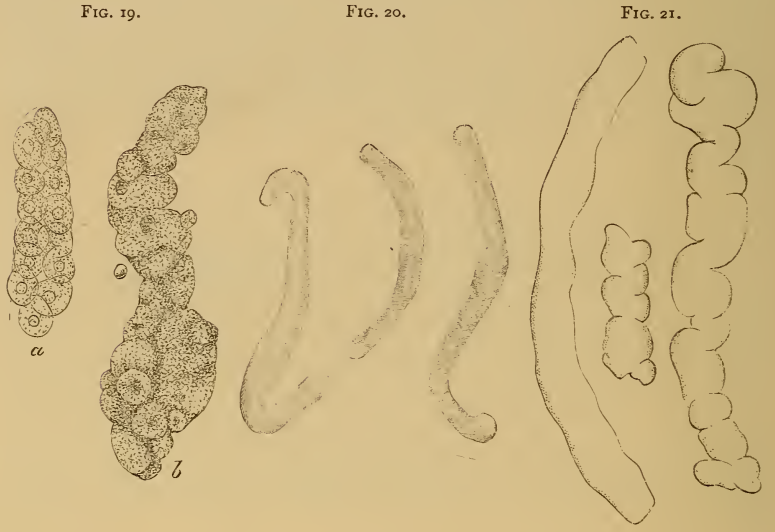
EPITHELIAL, ALBUMINOUS AND BLOOD CASTS.

The sediment of urine containing albumen generally contains small cylindrical fragments, which are formed in the uriniferous tubes, and hence are known as casts or tube casts. Besides blood casts, composed of blood corpuscles and fibrin, the result of hemorrhage, there are three kinds of casts, epithelial, hyaline and waxy. Epithelial casts are composed of the epithelial cells of the canals or tubes in which they are formed. The epithelial cells of these tubes are round-like bodies, represented by Fig. 23, page 79.

As the casts are made up of these cells, they are recognized by their dentated or irregular surfaces. In some of the casts the epithelial cells appear in rows, each having a well-defined outline. In other casts the rows are difficult to make out, and the outline or contour of each cell is indistinct; those of the latter class are known as metamorphosed epithelial casts. Both varieties are represented by *a* and *b*, Fig. 19. Epithelial casts refract light to a greater extent than the urine, hence with the microscope they are not difficult to find, as they appear bright with well-defined outlines. Epithelial casts are firmer in consistence, and resist the action of chemical reagents to a greater extent, than hyaline casts.

Hyaline casts (Fig. 20) are smaller and have smoother surfaces

than epithelial casts. They are not colored and refract light less than the urine, and are, therefore, more difficult to find with the microscope. Those that are somewhat long are usually bent, while the short ones are straight. Hyaline casts are somewhat granular, the granules being metamorphosed constituents of the uriniferous tubes. Waxy casts are the largest in size. They refract light to a much greater extent than the urine, so that in the field of the microscope they appear bright. They are represented by Fig. 21. They are slightly yellow in color. As casts readily disintegrate, especially in alkaline urine, the sediment is



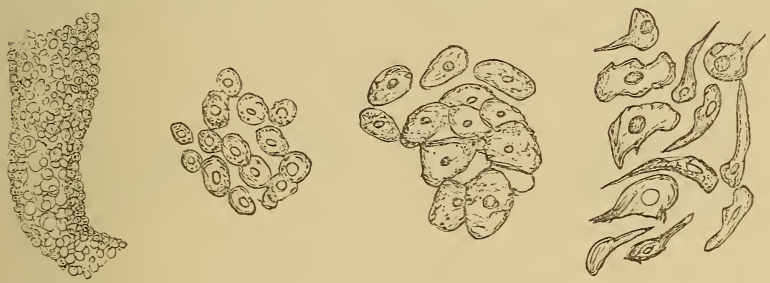
examined, if possible, as soon as it settles. In case of failure to find any casts in several preparations, filter some of the urine with sediment suspended, wash with a small quantity of water and transfer the sediment to a conical wine glass, and add a solution of either eosin or gentianin in quantity sufficient to impart a distinct color to the fluid, and after standing until the sediment subsides, it is examined with the microscope. The casts will be stained so as to become visible. Without having filtered, washed, or stained the sediment, and there is doubt as to the presence of hyaline casts, the process of staining may be carried on with the sediment on the slide (Rouvida). For this purpose,

water, in portions of one or two drops, is placed at the margin of the glass cover, and the fluid is absorbed from the opposite margin of the cover by means of slips of filter paper. Having thus washed the sediment, a small quantity of solution of gentianin is placed at the margin of the cover, and is drawn under the glass by a piece of filter paper, as in washing, when, in a short time, hyaline casts will become visible.

BLOOD CASTS.

Blood casts are formed of blood in the uriniferous tubes by the coagulation of its fibrin. They are, therefore, composed of red corpuscles and fibrin. They are represented by Fig. 22. As they appear in urine with blood, they are difficult to find, especially when the amount of blood is considerable. They are about the

Fig. 22. Fig. 23. Fig. 24. Fig. 25.



size of epithelial casts, but are dark, from the blood corpuscles they contain.

EPITHELIAL CELLS, BLOOD CORPUSCLES, PUS, SPERMATOZOA, BACTERIA, AND OTHER ORGANISMS.

Normal urine contains a few epithelial cells from the urethra, vagina of the female, ureters and pelvis of the kidneys.

By diseases of the mucous membrane the number of epithelial cells in the urine is increased, and in chronic inflammatory or degenerative processes, as in Bright's disease, amyloid and fatty degenerations, epithelial cells from the parts diseased are often metamorphosed, that is, contracted, and often contain particles of fat. Epithelial cells from the uriniferous tubes and urethra of the male differ from those of the pelvis of the kidney and bladder,

so that they may be recognized by the microscope. The epithelial cells of the uriniferous tubes are somewhat round and small (Fig. 23), while those of the pelvis of the kidney and bladder are flat and thin (Fig. 24). The epithelial cells of the neck of the bladder are irregular in shape (Fig. 25).

BLOOD CORPUSCLES.

Clots of blood may form in the urine after it has been passed, or they may form in the bladder and obstruct the passage of urine. Urine containing blood is generally cloudy and red or dark red in color, unless the quantity of blood present is small, when the urine is so changed in color that the presence of blood is suspected. In cases of hemorrhage of any part of the urinary passages not complicated by nephritis or cystitis, except at point of hemorrhage, the urine may be nearly normal, except that it contains blood. Generally, however, hemorrhages accompany acute inflammation of the kidneys, or they are produced by chronic ulcerative processes in the walls of the bladder, when the sediment contains pus and epithelial cells. The presence of blood casts in the sediment, a considerable quantity of albumen in the urine, and the absence of coagulæ of blood, not distinguishable without the aid of a lens or microscope, is evidence that the hemorrhage occurred in the kidneys. In urine containing blood or pus, fermentation begins much sooner than in normal urine, and as tube casts undergo changes in the presence of bacteria, by which they cannot be recognized, urine in which the presence of blood is suspected should be examined without delay. Blood corpuscles in the urine are often modified in form; sometimes they are nearly round, sometimes dentated, and again shrunken and apparently atrophied. Blood corpuscles, as they generally appear, are represented by Fig. 26. If in case of doubt of the presence of blood in the urine by microscopic examination, employ the spectroscopic test for coloring matters of the blood, page 61, or, not having a spectroscope at hand, employ Struve's test for hæmatin, page 63. For the tests for fibrin, refer to page 64.

PUS.

Pus cells, as they appear in the field of the microscope, are round-like bodies about twice the size of the red corpuscles of

the blood. They are represented by *a*, Fig. 27. The contents of pus cells are granular. They contain one or two nuclei, but on account of the granular matter they contain, the nuclei are not seen. By microscopic examination or by means of chemical reagents, mucus corpuscles cannot be distinguished from pus cells. In normal urine there are mucus corpuscles, but the number is limited. By irritation or inflammation of the mucous membrane of the urinary passages the number of mucus corpuscles in the urine is greatly increased. But at what period or stage of the disease pus cells appear in the urine is in some cases difficult to determine. If the inflammation is not confined to a small surface, but involves the mucous membrane of the bladder, the urine is turbid, often containing shreds of mucus, and when filtered the filtrate yields a light precipitate, mucine, by the addition of acetic acid. Besides pus, as found by microscopic examination of the sediment, the urine contains a small quantity of albumen, to determine the presence of which the urine is filtered,

Fig. 26. Fig. 27. Fig. 28.

and the filtrate rendered strongly acid with acetic acid, when it is filtered and the filtrate tested for albumen—refer to tests for Albuminous Bodies, page 46. If the quantity of albumen is considerable a part comes from the kidneys, and a careful examination of the sediment is made for tube casts. If pus comes from cystitis and the urine is alkaline from fermentation having taken place, crystals of magnesium ammon. phosphate (*a*, Fig. 29) and ammon. urate (*b*, Fig. 29) will be found in the sediment. In this condition of the urine the presence of pus cannot be determined with certainty by microscopic examination of the sediment, unless the urine has been alkaline but a short time. Alkalies change pus to a gelatinous mass, the cells dissolve leaving the nuclei. The contour of the cells is seen if the action of the alkali, ammon. carbonate in urine, undergoing fermentation, has not continued long. A similar change in pus cells takes place

by the action of dilute acetic acid (*b*, Fig. 27). The change in the cells is seen by placing a drop of the dilute acid at the margin of the glass cover, and by means of a piece of porous paper causing absorption of fluid from the opposite margin of the cover, when the acid will pass under the cover and come in contact with the cells. The disappearance of the walls of the cells with the granular matter is seen. The nuclei appear as dark, irregular fragments. The presence of pus in the sediment of urine, of acid reaction, is determined by stirring a piece of sodium hydrate with the sediment, when the pus becomes gummy and tenacious (Donné). In inflammation of the urethra of the male—gonorrhœa—the sediment of the urine contains pus in quantity, depending on the stage of the disease and frequency of passing the urine; but for the examination of urinary sediments containing pathological products of the urethra, small quantities of the urine first passed are collected in a conical wine glass, the sediment of which is examined.

Pus enters the urinary passages from abscesses in the pelvis, or parenchyma of the kidney resulting from obstruction, as stone, or from cancerous or tubercular formations. In these cases many pathological products may be found in the urine.

SPERMATOZOA.

Spermatozoa in the urine are found only by microscopic examination. To examine urine for spermatozoa, that passed first in the morning should be taken, and after standing several hours the sediment is examined by the microscope. The movements of spermatozoa soon cease in the urine, and especially if the urine is strongly acid in reaction. The general appearance of spermatozoa is shown by Fig. 28. They have a head and tail but no neck.

BACTERIA.

Normal urine when passed from the bladder and collected over mercury, having been boiled, will undergo no change for many months, providing the process is so conducted that no air comes in contact with the urine (Pasteur, Cazeneuve and Liron). By exposure to the air urine undergoes fermentation (page 20).

In urine having undergone fermentation in the bladder, as is frequently the case in inflammation of that organ, the micrococcus ureæ are found as in fermentation of the urine by exposure

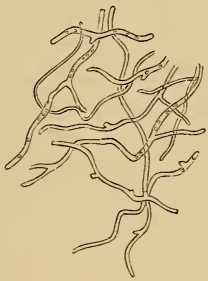
to the air. It is doubtful if the germs of bacteria are in normal urine not having come in contact with the air; but that they are often introduced into the bladder by the use of unclean catheters admits of no doubt, and in cases of chronic cystitis in which no catheter has been used, and yet fermentation of the urine takes place in the bladder, the germs may have found their way into the bladder by relaxation of the constrictor muscle of the urethra resulting from a diseased condition.

The micrococcus ureæ appear as thread-like bodies, some long, others short, and during life they are in continued movement. In alkaline urine they are generally found in the sediment with crystals of magnesium ammonium phosphate, ammonium urate and granular calcium and magnesium phosphates, as in Fig. 29.

FIG. 29.

FIG. 30.

FIG. 31.



By a high magnifying power the thread-like fragments are found to be made up of minute cells. By infectious diseases, as scarlet fever, diphtheria, erysipelas, etc., the urine is found to contain microörganisms. The kidneys, in the process of separating these organisms from the blood, often become inflamed, giving rise to albuminuria as an accompaniment of the disease. On the other hand, it is claimed that the albuminuria which is developed in these cases is due to diminished blood pressure in the kidneys, the result of high fever.

SARCINA AND OTHER MICROÖRGANISMS.

It is necessary that nutritive fluids be differently constituted for the culture of different microörganisms, so that the germs of those organisms for which the conditions of life are favorable

will germinate. It is probable that various forms of fermentation would take place in the urine while in the bladder in the course of infectious diseases, if the urine were so constituted that the different organisms excreted by the kidneys could be cultivated in it. Besides the micrococcus ureæ the sarcina are cultivated in the urine, yet the latter are rarely found in the urine. They differ from the sarcina sometimes found in the stomach in being about half the size. Urine containing sarcina is usually cloudy, and often contains a heavy sediment composed mostly of the organisms. Sarcina do not produce pathological changes in the mucous membrane of the urinary passages, neither is their presence in the urine associated with any general disease. In the sediment they are easily found by microscopic examination, as they differ from other organisms by being composed of cells of 4, 8, 16, 64 and sometimes 512 (Fig. 30). In the sediment of diabetic urine, cells of the ordinary yeast ferment are sometimes present. They are round-like bodies about the size of pus cells, but they have no nucleus and are usually in clusters. From pus cells they are distinguished by no nuclei appearing by the action of dilute acetic acid.

Of the moulds, the penicilium glaucum is found in urine more frequently than any other. When in urine, it usually appears on the surface, at first as a colorless film, and in time it becomes green or blue and more dense. By microscopic examination it is found to be composed of a meshwork of fine fibres—mycelium—as shown by Fig. 31, and with the fibres round cells, the spores, are generally found.

CHAPTER VI.

Scheme for the Qualitative Analysis of Healthy and Diseased Urine—Sediments—Microscopic Examination of Sediments—Staining—Scheme for the Qualitative Analysis of Sediments.

QUALITATIVE ANALYSIS OF HEALTHY AND DISEASED URINE.

In examining the urine in the order here given, frequent reference is made to the more elaborate consideration of the properties of each body, as well as to the methods employed in separating it from the urine, and identifying it in the preceding pages. This scheme embraces only those bodies in the urine a knowledge of which is of the greatest importance, and it is intended to serve the purpose of an index or guide in determining the quality of the urine, whether healthy or diseased. Following the qualitative examination of the urine will be found the scheme for analysis of urinary sediments, followed by methods employed in determining the constitution of concretions or stones.

Physical Properties.

Determine if the urine, when fresh and before it cools, if possible, is clear or turbid, and if clear, if a sediment forms as it cools. If fresh urine is turbid, determine if the sediment is amorphic or crystalline, and if crystals are present, their character—that is, if difficult to see without the aid of a glass, or if they are coarse (gravel).

Color.

Urine, highly colored, may be normal, but if so, it is concentrated, and for a given volume contains more urobilin than urine generally does. Urine of high specific gravity, from a person having fever, is highly colored; but diabetic urine, also of high specific gravity, is generally nearly colorless. If urine is highly colored, deep red or brown, examination for blood is made; if the color is dark yellow or green, and by shaking the urine a yellow green foam forms, the urine is examined for coloring matters of the bile.

Odor.

If the odor of the urine is well pronounced, determine if due to fermentation by microscopic examination for the micrococcus ureæ. The presence of an excess of ammon. carb. from fermentation gives to urine a strong urinary

odor. If the odor of urine is peculiar, it may be due to the ingestion of asparagus, turpentine, cubebs, etc. Determine the specific gravity of the urine by means of a urinometer. The following conditions for making this test accurately are taken into account. The markings of the scale should not be too close; the vessel holding the urine should be wide enough to permit the instrument to float without touching its sides; the temperature of the urine should be brought to that for which the instrument is graduated, and the instrument should be dried with a piece of cloth before using.

Specific Gravity.

If blue litmus paper changes instantly when brought in contact with the urine, it is strongly acid in reaction, and if red litmus paper turns blue at once, or yellow turmeric paper dark red, the urine is strongly alkaline in reaction. If the urine produces no change in the color of either blue or red litmus paper, it is neutral in reaction. Occasionally urine changes both blue and red litmus paper purple, owing to there being in solution a mixture of the neutral phosphates, which are alkaline in reaction, and the acid phosphates, which are acid in reaction. (Amphotic Urine of Bamberger.)

Chemical Properties.

If the urine is alkaline, examine for the micrococcus ureæ; if absent, the alkaline reaction is due to the presence of potassium or sodium carbonate.

Fat in the urine can scarcely be regarded as a sediment; if in the form of oil drops, the fat appears on the surface of the urine, and may possibly escape detection when the sediment is examined with the microscope. If, on the other hand, the fat is in the emulsified form, its presence would be discovered in examining the sediment with the microscope.

Fat.

In all examinations of the urine the surface should be carefully inspected to detect the presence of any floating particles of fat, or, if the urine by standing continues cloudy, a drop should be examined with the microscope to determine if fat globules or granules are present. (Refer to Fat in Urine, page 66.)

For ordinary clinical purposes the tests for serum albumen and globuline in urine are sufficient. It is only when special investigations in pathological conditions, which may be characterized by the appearance of more or less globuline, hemialbumose and peptone, that the presence of

Albuminous Bodies.

these bodies in urine is determined. Before testing for albumen, urine not clear should be filtered. Test for albumen with nitric acid, also with the sodium chloride and acetic acid test, page 46.

There are many other tests for albumen in urine, but these answer every purpose. For the method for testing for hemialbumose refer to page 47, for peptone page 47, and for globuline and serum albumen page 47.

Mucine.

Urine containing an abnormal quantity of mucine is usually turbid, and with an excess of acetic acid the turbidity is increased. To test for mucine, filter the urine through a double filter; dilute the filtrate with an equal volume of water and render strongly acid with acetic acid. A light precipitate forms if mucine is present.

Diabetic Urine.

Before testing for sugar, filter the urine, if necessary. Unless the urine contains a small quantity of sugar, there will be no difficulty in determining its presence by means of Salkowski's modification of Trommer's test (page 50) or with Fehling's solution (page 50); but when urine contains reducible substances in quantity sufficient to produce a yellow flocculent precipitate of cuprous hydrate, and doubt arises as to the presence of sugar, employ the fermentation test (page 52). With some experience in purifying salts by recrystallizing with but little loss, and determining the melting point of a body, the test for sugar in the urine in small quantities with phenylhydrazin (page 53) is practicable. Not having the fermentation tubes or phenylhydrazin at hand, coloring matter, etc., may be removed from the urine with either lead acetate or corrosive sublimate (page 51) and the filtrate tested for sugar with Salkowski's modification of Trommer's test and Fehling's solution. If the results should be unsatisfactory, separate the sugar from the urine by means of potassium hydrate or Bruecke's method (page 54) and test the aqueous solution for sugar.

Inosit.

To test for inosit, refer to page 55. Testing for this body may be omitted, except in cases of diabetes and Bright's disease.

Biliary Constituents.

For determination of the presence of biliary acids in urine refer to page 58, and for biliary coloring matters employ Gmelin's test, page 59.

Leucin and Tyrosin.

To test for leucin and tyrosin refer to page 65, but, unless there are symptoms indicating disease of the liver, the examination for the presence of these bodies may be omitted.

Indican.

In cases of chronic diseases of the intestinal canal, test for indican (page 27).

Urates.

Concentrate the urine after having filtered, if necessary, by evaporating on a water bath, render strongly acid with dilute hydrochloric acid, and after standing twenty-four hours uric acid will separate from the neutral urates. Filter, wash and test with nitric acid and ammon. hydr. (page 23).

Chlorine.

To determine the presence of chlorine combined with bases in the urine, render distinctly acid with nitric acid; add a solution of silver nitrate. A white precipitate forms if chlorine is present.

Phosphoric Acid of the Phosphates.

A part of the phosphoric acid combined with bases will precipitate by rendering the urine strongly alkaline with ammon. hydrate. The precipitate formed is composed of the basic phosphates of calcium and magnesium. To precipitate all of the phosphoric acid combined with bases, add ammon. hydrate to the urine until strongly alkaline, when an excess of a solution of calcium chloride is added.

Phosphoric Acid of Glycerin-phosphoric Acid.

To test for phosphoric acid combined in the form of glycerin-phosphoric acid, employ Sotnischewsky's method (page 34).

Sulphuric Acid combined with Bases.

Sulphuric acid combined with bases is precipitated by rendering the urine distinctly acid with acetic acid and adding a solution of barium chloride.

Sulphuric Acid in Ester Compounds.

To test for sulphuric acid in ester compounds, refer to page 36.

Ammonia.

For ammonia in combination, test by employing alcohol and a solution of platinum chloride (page 39).

URINARY SEDIMENTS.

It is evident that if urine contains a sediment, it is not soluble in the amount of urine in which it has formed. Some sediments form when the urine changes in reaction, and others, again, are produced by fermentation. In some cases the sediment is composed of organized elements of the tissues, or they are pro-

duced by pathological processes. It is therefore apparent that in the study of urinary sediments a number of data are taken into consideration. In collecting urine for examination, vessels absolutely clean are employed, otherwise fermentation of the urine may take place in a short time, or fat and other bodies be found in the urine which came from the vessel. The sediment will separate from urine in a bottle or glass cylinder so that it may be examined, but if the quantity is small, a conical wine glass is preferred. Before examining the sediment the reaction of the urine is determined, and also if the urine was turbid or clear when passed.

The principal part of the work of examining urinary sediments is microscopic in character, and the conditions for securing good results rest to a great extent on keeping clean the microscopic slides, glass covers, objectives, oculars—briefly stated, skill in working with the microscope, which, however, is not difficult to acquire.

The sediment having subsided, portions of it are drawn off by means of a glass tube about 25 cm. long, with internal diameter of four or five mm., and one end drawn out in the flame of a Bunsen's burner or spirit lamp, so that its orifice is about one millimetre in diameter. The upper end of the tube is fused in the flame of a blowpipe that its margins may be removed. By moistening the finger somewhat, and placing it firmly over the fused end of the tube, and sinking the drawn-out extremity into the sediment and partly removing the finger, air will pass out and some of the sediment with urine will enter the tube. The upper end of the tube is again closed and the sediment will remain in the tube, when a drop of the urine containing some of the sediment may be placed on a slide for microscopic examination, or a larger quantity introduced into a test tube by removing the finger. Some sediments subside sooner than others, and consequently form the lower strata of the deposit, so that it is well to draw off portions of the sediment for examination at different distances from the bottom of the glass. About one drop of the mixture of sediment and urine is sufficient to fill the space between the slide and glass cover when the latter is placed on the fluid. The quantity should not be sufficient to float the glass cover or pass over its margins. Some chemical reactions may

be made on the slide with the glass cover in place, more particularly those by which solution of solids takes place; but when crystals which form by chemical reactions are to be examined, the reaction, as a rule, is brought about before the glass cover is placed on the preparation. Some of the urine with sediment is transferred to a test tube, a few drops of the reagent added and mixed with urine by carefully agitating the test tube, and the solution having remained in quietude one or two hours, a drop of the fluid with crystals is placed on the slide by means of the drawn-out tube. Crystals formed in this way are generally more fully developed than when formed on a slide, as the solution may become concentrated by evaporation before the crystals have formed, and with the glass cover in place the space may be insufficient. For the formation of fully-developed crystals, space for the free movement of the molecules (hence solutions should be as dilute as the case will permit) and absolute quietude are conditions which cannot be ignored.

To ascertain if a sediment under examination dissolves when it comes in contact with a reagent, place a drop of the latter at the margin of the glass cover and bring a narrow strip of porous paper in contact with the fluid at the opposite margin of the cover; the reagent will pass under the glass cover and come in contact with the sediment. Care is taken that the current established is not sufficient to carry the bodies out of the field of view. To find hyaline casts in urinary sediments, it may be found necessary to employ a staining fluid by which they become visible. For this purpose, after the sediment has subsided, one or two cc. of the fluid containing some of the sediment is drawn off with a pipette and introduced into a small clean test tube, and a solution of eosin added in quantity to impart a distinct color to the fluid. Mix well by gently agitating the test tube, and in fifteen minutes transfer a drop of the fluid containing some of the sediment to a slide by means of the drawn-out tube. A solution of either picrocarmine or gentianin may be employed as well as eosin. These staining fluids may be purchased of any dealer in microscopic apparatus. The plan adopted here for examining sediments is to test for the urates, etc., in the sediment of urine decidedly acid in reaction, and for the phosphates of calcium and magnesium, etc., in urine strongly alkaline in reaction (refer

above to reactions of the urine), and if in this course other bodies are observed, reference is made to methods employed in finding constituents of sediments not depending on the reaction of the urine for their formation. As the greater number of urinary sediments do not depend on the reaction of the urine and cannot be separated into groups by reagents, any system for the analysis of them is necessarily defective.

(*A*) THE URINE IS STRONGLY ACID IN REACTION.

1. Heat some of the sediment with urine in a test tube—it clears up and reappears by cooling. Presence of the urates and possibly hippuric acid. The sediment appears to remain unchanged. Boil gently a short time, filter while hot and place filtrate one side for several hours—it becomes cloudy and by warming clears up. Presence of the urates. The sediment is, therefore, partly composed of urates.
2. By microscopic examination there is granular matter (Fig. 8, page 71), and when a drop of solution of sodium hydr. comes in contact with the granules, by placing it at margin of glass cover they dissolve. Presence of urates.
3. The crystals are large and tetrahedral in form (Fig. 10, page 71), soluble in ammon. hydrate. Presence of hippuric acid.
4. The crystals are well formed, four or six sided (Fig. 8, page 71). They remain unchanged with dilute hydrochloric or acetic acid, but dissolve in a solution of sodium hydrate. Presence of uric acid. Uric acid may be further tested by filtering the urine containing the sediment, washing with hot water, and testing the residue for uric acid with nitric acid and ammon. hydr. (page 23).
5. In the sediment there are crystals which are prismatic and needle-like in form, many radiating from a common centre (Fig. 11, page 72). They are insoluble in hydrochloric acid. Presence of calcium sulphate.

If, in the examination of the sediment of urine, strongly acid in reaction, by the methods here given, other bodies are observed by microscopic examination, to which no reference was made, examine them according to D and E; and if there is reason to believe that the urine is not sufficiently acid in reaction to pre-

vent the separation of the phosphates of metals of the alkaline earths, examine the sediment according to B, C and D.

(*B*) THE URINE IS STRONGLY ALKALINE IN REACTION.

1. By microscopic examination the sediment is granular or amorphic (Fig. 12, page 72). With acetic acid it dissolves. Presence of calcium and magnesium phosphates.
2. The crystals are large, well formed, prismatic, some having no corners, resembling in appearance a coffin lid (Fig. 13, page 72). The crystals are soluble in acetic acid. Presence of magnesium, ammon. phosphate.
3. The crystals are spherical, sometimes, though seldom dumb-bell in form (Fig. 17, page 76). The crystals dissolve in dilute hydrochloric or acetic acid, and gas bubbles are formed under the glass cover. Presence of calcium carbonate.

(*C*) THE URINE IS NEUTRAL, SLIGHTLY ACID OR ALKALINE IN REACTION.

1. By microscopic examination there are crystals wedge-shaped, having centres from which they diverge (rosettes), Fig. 14, page 74. The crystals are soluble in dilute acetic acid and insoluble in a solution of sodium hydrate. Presence of calcium neutral phosphate. With this sediment there may be bodies described under A, B and D.

(*D*) CRYSTALLINE BODIES ARE IN THE SEDIMENT NOT DEPENDING ON REACTION OF THE URINE.

1. By microscopic examination the crystals are spherical, some having thorn-like outgrowths (as wild gooseberries), Fig. 15, page 74. When treated with a drop of acetic acid they disappear, but crystals of uric acid form after the lapse of some time (Fig. 9, page 71). Presence of ammon. urate.
2. The crystals are small,octahedral, with lines crossing (envelope form), Fig. 16, page 74. Occasionally they are in the form of dumb-bells. In dilute hydrochloric acid they dissolve, but are insoluble in acetic acid. Presence of calcium oxalate.
3. The crystals are six-sided and tabular (Fig. 18, page 76). The crystals undergo no change with acetic acid but dissolve in ammon. hydrate. Presence of cystin.

4. The crystals are needle-like and radiate from centres (rosettes), *b*, Fig. 5, page 65. The crystals are soluble in ammon. hydrate. Presence of tyrosin. Test the urine for leucin and tyrosin, page 65.

(*E*) ORGANIZED BODIES—PATHOLOGICAL PRODUCTS—ARE IN THE SEDIMENT.

1. By microscopic examination they are cylindrical bodies, having irregular but well-defined margins (*a*, Fig. 19, page 78). The cell formation of some is seen as shown by *a*, Fig. 19, while in others the surfaces are more homogeneous or agglutinated as seen by *b*, Fig. 19. Presence of epithelial casts. With dilute acetic acid they undergo no change. Those represented by *b* are metamorphosed.
2. They are cylindrical in form, smaller than those of E 1, surfaces smooth; contents homogeneous or granulated, with outlines so indistinct that they are seen with difficulty (Fig. 20, page 78). Presence of hyaline casts. They dissolve slowly in acetic acid, and with iodine dissolved in a solution of potassium iodide they turn yellow. For the employment of staining fluids in microscopic preparations, refer to page 90.
3. They are cylindrical in form, larger than those of E 1 or 2; contents amorphic gray or somewhat yellow in color, and more easily seen than those of E 2. Some are even in outline, while others are tortuous (Fig. 21, page 78). Presence of waxy casts.
4. They are cylindrical in form, dark in color, formed of round-like bodies (Fig. 22, page 79). Presence of blood casts. Examine the urine for coloring matters of the blood with the spectroscope (page 60) or by Struve's test (page 63), and for blood corpuscles according to E 5.
5. They are disc-like, round, dentated, or serrated bodies (Fig. 26, page 81). Presence of red corpuscles of the blood. Test the urine for coloring matters of the blood with the spectroscope (page 60), also test for hæmatin according to Struve, page 63.
6. They are round-like bodies, each having a nucleus. In size they are larger than pus corpuscles (Fig. 23, page 79). Presence of epithelial cells of the uriniferous tubes of the kidney

or of the urethra of the male. If the cells are somewhat numerous they arise from an inflammatory condition of the parts.

Epithelial cells are sometimes granular and contracted, and sometimes contain particles of fat, the result of chronic disease.

7. They are large, flat, nucleated bodies (Fig. 24, page 79). Presence of epithelial cells of the bladder.
8. They are round-like bodies with granular contents (*a*, Fig. 27, page 81), which with dilute acetic acid form imperfect nuclei (*b*, Fig. 27). Presence of pus corpuscles (as pus corpuscles cannot be distinguished from mucus corpuscles, the presence of the former is determined by their number).
9. They are masses of organized bodies, some cellular and some fibrous, with pus and epithelial cells. Presence of pathological products. Refer to page 82.
10. They are exceedingly small bodies, and unless high powers of the microscope are employed (1000 diameters), they appear as mere points, but in continued movement. In fresh urine with high magnifying powers (1200 to 1800 diameters), the bodies are found to be elongated, formed of two or more cells. In urine, three or four days after having been passed, they appear as round cells (Fig. 29, page 83). Presence of the micrococcus ureæ. (Refer to Fermentation of the Urine, page 20.)
11. They are cells in numbers of 4, 8, 16, etc. (Fig. 30, page 83). Presence of sarcina.
12. They are filamentous organisms, sometimes found in movements, each with head and tail but no neck or body (Fig. 28, page 81). Presence of spermatozoa.
13. They are masses of fibres and sometimes accompanied by round cells (Fig. 31, page 83). Presence of the ordinary mould penicilium glaucum.

CHAPTER VII.

Concretions or Stones in the Bladder—Constitution and Physical Properties of Concretions—Some of the Causes of the Formation of Concretions—Scheme for the Analysis of Concretions or Stones.

CONCRETIONS OR STONES IN THE BLADDER.

The following constituents of the urine enter into the formation of concretions in the bladder, pelvis of the kidneys or ureters. Uric acid with the urates, calcium and magnesium phosphates, magnesium ammon. phosphate, calcium oxalate, cystin, and xanthin. Of 545 stones, according to Ultzmann, 80.9 per cent. was uric acid, 8.6 per cent. phosphates, 5.6 per cent. calcium oxalate, 1.4 per cent. cystin and 3.3. per cent. foreign bodies introduced into the bladder or coagulæ of blood. Generally, several of these bodies are in a concretion. Occasionally, however, either cystin, calcium oxalate or ammon. urate is the only constituent. The broken surfaces of a concretion formed of different constituents, present a stratified appearance, due to layers or strata of the constituents. Concretions differ very much as regards color, consistence and other physical properties. Those composed of uric acid and urates are hard and dark red in color. Those composed chiefly of the phosphates are soft and white. Those composed of calcium oxalate are exceedingly hard, white and, when small, have smooth surfaces, and as they increase in size their surfaces become irregular. If ammon. urate is the principal constituent they are soft and light yellow in color. The formation of a concretion in the bladder is due to crystallization, and the conditions favoring the crystallizing process here are the same as they are in a beaker glass. Gravel or a sediment formed in the bladder, the crystals of which are visible without the aid of a glass, generally precedes the formation of concretions, consequently crystals or clumps of crystals, or masses of amorphic matter as calcium and magnesium phosphates, often become the nuclei of concretions when by their absence there would be no danger of the formation of stones or concretions. Not only do crystals or amorphic matter in the bladder often lead to the formation of

stone, but coagulæ of blood in the bladder in case of hæmaturia, and foreign bodies introduced into the bladder are known in many instances to have caused the formation of stone. It is of great importance in cases of gravel, when fresh urine contains free uric acid or there is present the phosphates of calcium and magnesium due to alkaline reaction of the urine, that the condition of the blood be so changed by diet or medication that the urine becomes free of gravel or any cloudiness while in the bladder, to avoid one of the conditions favoring the formation of concretions.

QUALITATIVE ANALYSIS OF CONCRETIONS OR STONES.

(*A*) After having broken, powder some of the fragments and heat a small portion of the powder on a platinum foil or in a small porcelain dish over a lamp to dull redness; continue the heat several minutes. It burns completely, leaving no residue.

Presence of cystin, urate of ammonium, uric acid, or xanthin, continue according to A 1. It chars or blackens, leaving a residue, pass on to B 1.

1. Separate the uric acid, either free or combined, if present, from the other constituents by treating some of the powder of the stone with an excess of dilute hydrochloric acid in a small flask; warm some time on a water bath. A residue is left. Presence of uric acid. Filter while warm, wash with water and transfer the residue to a small porcelain dish, treat with nitric acid and evaporate to dryness on a water bath. To the residue add a small quantity of ammon. hydrate. If a dark purple color is produced, it is proof of the presence of uric acid.
2. Evaporate the filtrate of A 1 to dryness on a water bath and mix the residue in a small beaker glass with calcium hydrate; add enough water to make a thick paste. Over the beaker place a watch glass, to the under surface of which pieces of yellow turmeric and red litmus paper are attached by having been moistened with water. At ordinary temperatures, or by gently warming the mixture, the yellow paper turns dark red or brown, while the red paper turns blue. Presence of ammonia.
3. Treat some of the powder of the concretion with water ren-

dered strongly alkaline with ammon. hydrate; filter, and to the filtrate add a solution of silver nitrate. If a precipitate forms, filter and wash with water containing some ammon. hydrate, transfer the precipitate to a flask, mix with water and separate the silver with sulphureted hydrogen gas. Filter and evaporate the filtrate with wash water, in a small evaporating dish on a water bath, to dryness. Treat the residue with some strong nitric acid, warm gently and add a solution of sodium hydrate. A dark red color is evidence of the presence of xanthin.

4. Triturate some of the powder of the concretion with water rendered strongly alkaline with ammon. hydrate, filter into a small beaker glass and wash the residue with water containing some ammon. hydrate. Evaporate the filtrate with wash water in the small beaker over wire gauze to near dryness. If crystals form, which by microscopic examination resemble those represented by Fig. 18, page 76, cystin may be present. Dissolve some of the crystals in a solution of sodium hydrate and add a small quantity of a solution of sodium nitroprusside. If a purple color is produced, cystin is known to be present.

(*B*) When some of the powder of the concretion is heated on a platinum foil or in a small porcelain crucible, it chars or blackens, and leaves a gray-like residue, showing the presence of calcium and magnesium phosphates or calcium carbonate from the oxalate.

1. Separate the uric acid, if present, and test for it according to A 1. Preserve the filtrate for testing according to B 3 and 4.
2. For xanthin and cystin, separate each from powder of the stone and test for each according to A 3 and 4.
3. Evaporate half of the filtrate of B 1 to dryness on a water bath, and examine the residue for ammonia according to A 2.
4. To the other half of the filtrate of B 1 add ammon. hydrate to alkaline reaction, warm, and after the lapse of a few hours filter, and wash with water rendered strongly acid with acetic acid (to dissolve the phosphates) until the wash water is free

of chlorine, known by producing no precipitate or becoming turbid when tested with a solution of silver nitrate, having been rendered acid with nitric acid. Dry the filter with precipitate and transfer the latter to a small platinum crucible, and heat carefully until the bottom of the crucible becomes dull red in color. During the application of heat the crucible should be covered with a lid. When cold transfer the residue in the crucible to a test tube and treat with dilute hydrochloric acid, and warm gently. It effervesces. Presence of calcium oxalate. By heat the oxalate is changed to the carbonate with the liberation of CO, and the carbonate so formed is decomposed by the acid with the escape of CO_2. By the decomposition of a minute quantity of calcium carbonate with hydrochloric acid in a test tube, the escape of the gas may not be detected.

The reaction is made in the field of the microscope by placing some of the carbonate on a slide, mixing with a drop of water, and when the glass cover is in place, a drop of acetic acid is brought to the margin, and by absorbing water from the opposite margin of the cover with a piece of porous paper, the acid will come in contact with the carbonate, and gas bubbles will be seen evolving in the field of the microscope as decomposition takes place.

5. Incinerate some of the powdered concretion or stone in a platinum crucible at a red heat; when cold treat with dilute hydrochloric acid; filter, wash the residue, if any, and unite the filtrate and wash water containing the calcium, magnesium and phosphoric acid. Treat the acid solution with a solution of sodium carbonate until nearly neutral, or slightly acid in reaction, when a solution of ferric chloride is added until a drop of the fluid, by means of a glass rod brought in contact with some ammon. hydrate in a porcelain dish, will produce a buff-colored precipitate. The formation of a dirty white precipitate before the buff-colored precipitate appears, indicates the presence of phosphoric acid. Add to the solution an excess of a solution of sodium acetate, heat to the boiling temperature, and filter while hot, and wash with water containing some sodium acetate. The filtrate contains the calcium and

magnesium, which examine according to B 6. Transfer the precipitate containing the phosphoric acid, if present, to a flask, and boil with a small quantity of a solution of sodium hydrate (1 part to 10 parts of water). Dilute with water and filter. The filtrate contains the phosphoric acid, if present, combined with sodium, which is tested with magnesia mixture. (A solution of magnesium sulphate treated with an excess of ammon. hydrate and the precipitate dissolved with a solution of ammon. chloride.) A white crystalline precipitate forms at once, especially if the solution contains an excess of ammon. hydrate, or it forms after the lapse of a few minutes. Presence of phosphoric acid.

6. The filtrate of B 5, obtained by filtering after treating with ferric chloride and sodium acetate and boiling, is rendered distinctly acid with hydrochloric acid, and boiled to drive off any carbonic acid that may be present. Test a portion of the solution in a test tube with a solution of potassium ferrocyanide. It should give no precipitate, showing absence of ferric chloride, and when another portion in a test tube is rendered alkaline with ammon. hydrate, and an excess of a solution of ammon. chloride added, no precipitate should be produced, showing absence of phosphoric acid. If ferric chloride and phosphoric acid are absent, the filtrate is rendered distinctly alkaline with ammon. hydrate and an excess of a solution of ammon. chloride added.

   The solution is then heated to the boiling temperature and a solution of ammon. oxalate added in excess; a white precipitate is produced. Presence of calcium. Filter, and test the filtrate for magnesium according to B 7. If ammon. oxalate produces no precipitate, hence absence of calcium, the fluid is tested for magnesium, according to B 7, without filtering.

7. The filtrate from calcium oxalate, B 6, is free of calcium if no precipitate forms by the addition of a solution of ammon. oxalate; and if the calcium has been separated, or no calcium was found, the filtrate or solution is rendered strongly alkaline with ammon. hydrate, and a solution of sodium ammon. phosphate added; a white crystalline precipitate is produced.

Presence of magnesium. With the methods here employed tests for sulphuric and carbonic acids are not given, neither are the tests for potassium and sodium, as they are in mere traces in stones or concretions, and therefore their presence or absence is not considered in classifying stones or concretions.

CHAPTER VIII.

Filter Paper and Filtering—Evaporating—Drying—Ashing Filters and Heating Precipitates—Chemical Balance and Weights—Weighing—Vessels Required for Measuring Fluids—Desiccators, Tongs, Crucibles, etc.—Preparation of Solutions for Volumetric Analysis—Normal Oxalic Acid—Normal Potassium Hydrate—Normal Sulphuric Acid—Normal Hydrochloric Acid—Solution of Litmus—The Barium Mixture—The Magnesia Mixture—Millon's Reagent.

QUANTITATIVE ANALYSIS.

FILTER PAPER AND FILTERING.

In quantitative work Schleicher and Schüll's filter paper, No. 589, is used with advantage, as it leaves no ash by burning. No. 590 of Schleicher and Schüll is finer paper, and is preferable in filtering and washing finely-divided precipitates, as barium sulphate. Either variety of paper is procured, cut in circular pieces. The sizes usually employed are 5½, 7 and 9 cm. in diameter. Swedish filter paper, bearing the name of J. H. Munktell in water letters, is the finest filter paper, but it is sold in sheets and leaves an ash by burning. To remove the mineral substances from Swedish filter paper it is cut in circular pieces by means of a lathe, when they are folded in quadrants and placed in a beaker glass in order that they may retain their form, and then treated with dilute hydrochloric acid—1 part acid to 6 parts water. After remaining in contact with the acid a few minutes the latter is drained off, and the filters washed with distilled water by standing in contact with them a few hours and draining off. The process is repeated until the last trace of hydrochloric acid disappears from the wash water, known by testing with a solution of silver nitrate. The filters are then dried by placing them between porous paper and kept in a warm place. Swedish filter paper when treated in this way is practically free from ash. In regard to the process of filtering, funnels having an angle of 60°, with even surfaces, are employed, as the paper comes in contact with every part of the surface and is thereby supported. The margin of the paper, when fitted in the funnel, should be about 1 cm. of the brim of the funnel. Before filtering, the paper is moistened with water and the

filter pressed to the glass, so as to exclude all air bubbles, as their presence interferes with the washing. If a fluid composed mostly of alcohol is to be filtered, the filter is moistened with alcohol instead of water. Each time the fluid is put into the filter, when filtering or washing, the amount should not be sufficient to fill the filter, as some of the precipitate may pass over the margin of the paper and lodge between it and the funnel. At first the clear fluid is decanted into the filter, as it passes through the paper more rapidly than after the precipitate is brought on the filter. To avoid loss, the fluid is introduced from the beaker or dish into the filter by means of a stirring rod, placed at the lip of the beaker or dish, and the fluid passes down the rod into the filter. In washing a precipitate, before each addition of fluid to the filter, any fluid already in the filter is allowed to pass through. In transferring the precipitate from a beaker glass or dish, a stirring rod, over the end of which is placed a short piece of rubber tubing, is employed. The rubber will answer the purpose of loosening the precipitate from the beaker or dish. To facilitate the process of filtering, a filter pump may be employed, or, in the absence of which, an aspirator bottle containing water will answer the purpose. A platinum cone fitted into the funnel, in which the folded end of the filter is placed, will support the paper if the cone and paper be properly adjusted.

EVAPORATING.

Generally, solutions are concentrated by evaporation on a water bath, yet if the fluid contains no bodies mechanically suspended, it may be evaporated over the free flame with the heat so regulated that the fluid will not reach the boiling point. Alcohol and ether solutions are evaporated on a water bath at a low temperature. In the evaporation of fluids for the purpose of procuring crystals for microscopic examination, the solutions should be filtered, and the process carried on slowly by placing near a stove or steam pipes, or if a good air pump is at hand, by removal of the atmospheric pressure; especially over concentrated sulphuric acid, evaporation will be greatly facilitated.

DRYING.

In estimating organic substances gravimetrically, as, for example, uric acid, kreatinin and albumen, the weight of the substance is ascertained by drying the filter paper in an air bath at a certain temperature, usually at 100° C., between two watch glasses held in place by a wire spring, and after cooling in a desiccator the filter paper, with the watch glasses, is weighed. In order that the paper may contain no moisture, the heating and weighing are repeated until the weight becomes constant. After filtering and washing, the paper and precipitate are dried and weighed as before, and the increase in weight is the weight of the substance. Bodies that are perfectly insoluble in alcohol and ether may be washed with a mixture of alcohol and ether after having been washed with water. By this treatment precipitates will dry much sooner than by washing with water alone, but in exact work the filter should be washed with a mixture of alcohol and ether before drying and weighing, as there is some loss in weight by the action of alcohol and ether. An air bath is a box of sheet iron or copper, provided with a door and shelf or triangle support, on which the watch glass or dish containing the substance is placed. A thermometer is introduced into the bath through an opening in the upper wall.

ASHING FILTERS AND HEATING PRECIPITATES.

When the filter and precipitate are thoroughly dry, the latter is transferred from the paper to a clean dry watch glass, and the paper is folded a few times, and with a platinum wire it is surrounded by three or four turns, when the filter paper is burned by holding it in the oxidizing zones of a Bunsen's flame or spirit lamp. Any particles of the precipitate that may have fallen on the glazed paper or burner plate over which the operation is conducted, are collected in the watch glass with a hair pencil. When the ash is free of carbon the platinum wire is held over the precipitate and removed from the wire with the hair pencil. If in the precipitate there is a reducible metal, as, for example, silver in the chloride, loss would likely attend the process, as the metal would form an alloy with the platinum wire. Instead of ashing on the wire the paper is folded, placed in the weighed crucible

supported by a triangle, and heated gradually until the paper ceases to burn with the production of a flame when the crucible is heated to redness and usually in fifteen minutes the paper will be completely ashed. By inclining the crucible somewhat, air will enter in greater quantity. Instead of ashing the paper in the crucible the lid of the crucible may be employed for the purpose, by placing it on the support, with its concave surface upward. In case the precipitate is light and small in quantity, it may remain with the paper during the process of ashing. To prevent loss during the first part of the process, the filter paper with precipitate is placed in the crucible closed with the lid, and heat is gradually applied until the paper ceases to burn with a flame, when the heat is continued with the open crucible until the carbon of the paper disappears by oxidation. Any carbon that may have been deposited on the under surface of the lid is removed by heating.

CHEMICAL BALANCE AND WEIGHTS.

A balance of precision which will bear 100 grammes in each pan, and sensitive to 0.1 milligramme, will answer for all weighing required in urinary analysis. The requisites of a good balance are given in any modern work on physics. The weights required are 50 grms. down to 1 milligrm., with two 10 milligrm. riders. Weights, regardless of the reputation of the maker, should be tested before using. A set of weights may be too light or too heavy, and the results of quantitative work be correct as far as the weights are concerned, that is, the 0.5 grm. piece may not be $\frac{5}{1000}$ of 1 grm. and the 0.05 grm. piece may not be $\frac{5}{10000}$ of 1 grm.; yet, if the relative weights are as represented and the weights of the set be employed in all weighings, the relative quantities weighed would be the same; therefore, in testing a set of weights, standard weights, or a standard 5, 10 or 20 grm. piece, need not necessarily be employed. However, in preparing some solutions for volumetric work, errors would arise by employing a set of weights not absolutely correct, as 50 cc. water measured would not weigh 50 grms. by the weights. To determine if the weights of a set bear correct relationship, place on one pan of the balance, when adjusted, the two grm. piece and on the other pan two of the three one-grm. pieces, and ascertain if they are of equal weight. Now

substitute for one of the grm. pieces the third grm. piece and determine if they equal in weight the two-grm. piece. Return the weights to the case and place on one of the pans the five-grm. piece, and on the other pan the two and three one-grm. pieces, and test as before. The higher weights and the deci- and centigrm. weights, with the centigrm. riders, are tested in the same way. In making these tests exact differences are determined by employing one of the riders on the arm of the beam with the lighter weight. The weights of less than one grm. should not differ more than 0.1 milligrm., and the weights of more than one grm. should not differ more than 0.2 milligrm.

WEIGHING.

A fine balance of precision should be protected from hydrogen sulphide, nitrous fumes and dust. While weighing, every movement should be guarded that the balance receive no jar; and when the beam is raised from the knife edges or lowered, so as to rest on them, the movement should be made with the greatest care, to avoid rapid oscillating movements of the beam. The balance is in equilibrium when the beam oscillates equally, known by the needle leaving the 0 point an equal number of spaces each oscillation, and when the beam ceases to oscillate the needle points to the 0 point. The balance is brought in equilibrium by an adjustment screw at the end of each arm of the beam. When the balance is not in use the beam is raised from the knife edges. Usually, the body to be weighed is placed on the left pan, but not until the beam is raised and the pans arrested. The weights are handled with pincers, and under no circumstances with the fingers, and when the weights are not in use the case containing them is closed. The placing of the weights on the right pan is done in order. This will be understood by illustration. Suppose the weight of a crucible is 12.5322 grms. The 20-grm. piece is found too heavy, next try one of the 10-grm. pieces—too light—let it remain and try the 5-grm. piece—too heavy—return it to the case and try the 2-grm. piece—too light—let it remain and try one of the three 1-grm. weights—too heavy—return it and try the 0.5-grm. platinum piece, and so continue until the additional weight required is less than 0.010 grm., when the rider is placed

on the beam at number 5, and being found too heavy in this case it is moved to 2.5 or 3, and so on until the rider is so located on the beam that the latter is in equilibrium. To avoid mistakes in recording the weight, first note the weights absent from the case, and when they are returned to the case they are again noted, and when the numbers are compared, if a mistake has been made, it is discovered. In the example given, it is found that the weights absent from the case are the 10- and 2-grm. pieces, with the 0.5 and 0.03-grm platinum pieces, and on the beam the rider is on the first mark to the right of number 2; hence, the weight is 12.5322 grms. Bodies are never weighed while warm.

VESSELS REQUIRED FOR MEASURING FLUIDS.

A Measuring Flask has a flat bottom and long neck, on the lower part of which it is graduated by a mark. They are of various sizes, but those generally required are for measuring one litre (1000 cc.), one-half litre (500 cc.), one-fourth litre (250 cc.) and 100 cc.

Glass Cylinders are provided with a base and glass stopper, and graduated, the numbers increasing from below upward.

A cylinder may be employed in measuring any quantity to the amount for which it is graduated, that is, with a litre cylinder, 480, 510, or any other number of cc. within 1000, may be measured, but as the diameter of a cylinder is comparatively great, a small quantity of fluid making no appreciable difference in reading the meniscus of the fluid, it cannot be employed where great accuracy is required. Cylinders for measuring 100, 250, 500 and 1000 cc. are usually employed. A cylinder of 2000 cc. is convenient for measuring the quantity of urine formed in twenty-four hours.

Pipettes serve the purpose of transferring a definite quantity of fluid from one vessel to another. They are of different sizes, according to whether they are to measure a definite quantity, for example, 5, 10, 20 or 50 cc. for each quantity, employing a pipette graduated for the purpose. There are also pipettes, graduated as burettes, for measuring from 0.1 cc. to the maximum number of cc. for which the pipette is graduated.

Burettes of 30 and 50 cc. are generally employed. Each

space representing 1 cc. is graduated in fifths or tenths. The inferior extremity of a burette is connected with the tip or dropper by a piece of vulcanized rubber tube, which is secured by a Mohr's pinch-cock, to regulate the quantity of fluid in titrating. This piece of apparatus is preferable to a glass stop-cock, but a short piece of glass rod, with its ends rounded by heating in the flame of a blowpipe, placed in the rubber tube will answer every purpose, if it fits the tube close enough to prevent the passage of fluid. By pressing the sides of the rubber tube with the finger and thumb it will separate from the plug, unless the latter be too large, and the fluid will pass through the openings. If, after a burette or pipette has been washed with water, and dried, drops of the fluid it is intended to measure adhere to its sides, it should be well washed with alcohol and ether before using.

The burette is filled by placing the tip, or dropper, in the fluid, and while the pinch-cock is loosened by pressure, the fluid is drawn up by suction in quantity sufficient to fill the lower end of the burette, when the rubber tube is secured, and a small funnel is placed in the upper end of the burette, and the fluid is carefully poured into the burette until it stands but a short distance above the 0 mark. The fluid is now allowed to pass out of the burette drop by drop, by pressing the pinch-cock until the meniscus of the fluid coincides with the 0 mark. By the use of Erdmann's float readings are made with greater precision. In reading the meniscus of a fluid in a burette, sufficient time should elapse before the reading, so that the fluid adhering to the surface may run down; the eye should be on the same plane as the meniscus of the fluid, and all readings should be conducted on the same basis; that is, if the background is light, and the dark line below the light segment is taken as the meniscus in the first reading, it should be taken in all subsequent readings.

Measuring vessels, whether flasks, pipettes or burettes, should be tested before using. They are graduated to measure fluids at 17.5° C. As 1 cc. distilled water at this temperature weighs 1 grm., pipettes and burettes are tested by weighing a measured quantity of distilled water on a balance of precision. For this purpose, balance a beaker glass with a watch glass containing shot and tin foil, and into the beaker introduce a measured quan-

tity of water at 17.5°. In testing burettes a weighing is made for every 10 cc. If in weighing 10 cc. water the weight comes within 10 milligrms. of 10 grms., the burette may, according to Fresenius, be considered correctly graduated. If, however, an Erdmann's float be employed, the difference should not exceed 2 milligrms. In testing graduated flasks of more than 100 cc. a balance which will bear the increased weight and show a difference of 0.1 milligrm. is required.

DESICCATORS, TONGS, CRUCIBLES, ETC.

Of the various forms of desiccators that of Fresenius, having a capacity of 1500 cc., is preferable, as a crucible is placed in or taken out by means of the tongs without obstruction. By the application of some grease to the ground surfaces of the lid, and employing concentrated sulphuric acid as the dryer, the apparatus answers the purpose. Brass tongs are kept clean without much trouble, and do not rust or corrode, as iron. They should be bent so as to transfer crucibles without inconvenience.

The Royal Berlin Porcelain Crucibles are the best quality, being thin, light, well glazed, and resist sudden change of temperature without breaking. Those having a capacity of 14 cc. are convenient for ashing filter paper, heating precipitates, and weighing.

A platinum crucible of 20 or 22 grms., including weight of cover, and capacity of 22 cc., will answer every purpose in urinary analysis when a platinum crucible is preferred. A platinum dish, with cover, weighing 40 to 45 grms., having a capacity of 50 cc., is a convenient size for estimating the chlorides of potassium and sodium.

Other articles required, but not enumerated here, are: a burner plate, over which to burn filters; a piece of platinum wire, No. 5, on which to burn filters; a sheet of glazed paper, a pair of small pincers and a fine hair brush or pencil.

PREPARATION OF SOLUTIONS FOR VOLUMETRIC ANALYSIS.

The system of standardizing solutions employed in volumetric analysis is based on the valence of one atom of hydrogen. Of a monobasic acid, its molecular weight in grammes occupying the volume of 1000 cc. is normal. The molecular weight of nitric

acid, HNO_3, is 63. Therefore, 63 grms. pure nitric acid diluted with water to 1000 cc. is normal acid. The molecular weight of potassium hydrate, KOH, is 56; hence, 56 grms. in 1000 cc. is normal potassium hydrate. The molecular weight of sodium hydrate is 40; hence, 40 grms. in 1 litre. It is understood that one-half of the molecular weight of a dihydric acid or a dibasic oxide in grammes, dissolved in water and diluted to the volume of 1000 cc., has equal chemical value to the full molecular weight of a monohydric acid or monobasic hydrate in grammes occupying the volume of 1000 cc. In case a compound contains water of crystallization, the molecular weight of the water is added to that of the compound; for example, oxalic acid has the formula—

$$C_2H_2O_4,2H_2O.$$

The molecular weight of the acid proper, $C_2H_2O_4$, is 90, and that of the water, $2H_2O$, is 36. $90 + 36 = 126$. 126 is therefore the molecular weight of the acid, and as this acid is dibasic, ½ of 126 in grammes, 63, occupying the volume of 1000 cc., is normal. In standardizing solutions of alkalies and acids, pure oxalic acid may be employed. To avoid error, however, the acid marked chemically pure is dissolved in hot water to saturation, filtered while hot, and the filtrate stirred with rapidity in a beaker surrounded by cold water until the temperature is reduced. Fine crystals will separate. Filter, and wash the crystals on the filter with a small quantity of cold water and place between porous paper. Commercial oxalic acid is purified by recrystallizing several times.

NORMAL OXALIC ACID (63 grms. $C_2H_2O_4,2H_2O$ in 1000 cc. WATER).

Pure oxalic acid is placed between filter paper and pressed until dry, when about 20 grms. are weighed on a balance of precision and introduced into a clean, dry flask. The quantity of water in which to dissolve the acid is determined by the equation 63 : 1000 : : weight of oxalic acid : x. x = No. of cc. water. The solution will undergo no change if heated to near the boiling point in a flask which is closed with a rubber stopper while the solution is hot, and kept in a cool, dark place.

NORMAL POTASSIUM HYDRATE (56 grms. KOH in 1000 cc. WATER).

Potassium hydrate is preferred to sodium hydrate on account of the tendency of the latter to etch the burettes. As either com-

pound "chem. pure" usually contains more or less of the carbonate, the latter is separated by treating a solution with barium hydrate until after mixing well by shaking a small quantity, filtered into a test tube containing a solution of barium hydrate, will produce no cloudiness. Another portion is filtered, and the filtrate tested with dilute sulphuric acid, and if a light precipitate or cloudiness is produced, barium hydrate has been added to the solution of potassium hydrate in excess, to separate which add small quantities of a solution of potassium carbonate, mixing well by shaking after each addition. The solution is thus treated with barium hydrate and potassium carbonate until small portions, filtered, will produce no cloudiness with either barium hydrate or dilute sulphuric acid. A solution of potassium hydrate or sodium hydrate may be prepared by dissolving 70 grms. of the former or 50 grms. of the latter in 1100 cc. water; and after separating the carbonic acid, titrating with the normal oxalic acid as below, or a larger quantity of a much stronger solution may be prepared; and having separated the carbonic acid from the solution (employing a solution of sodium carbonate to separate excess of barium hydrate from the sodium hydrate solution), when the solution is diluted for titration, by first determining its specific gravity with a hydrometer from which the per cent. of either KOH or NaOH is ascertained by consulting Table 2 or 3.

TABLE 2.—SHOWING PERCENTAGE OF KOH IN AQUEOUS SOLUTIONS CORRESPONDING TO THE SPECIFIC GRAV., TEMP. 15°. (*LUNGE.*)

SPECIFIC GRAVITY.	PERCENTAGE.	SPECIFIC GRAVITY.	PERCENTAGE.	SPECIFIC GRAVITY.	PERCENTAGE.
1.060	7.4	1.142	16.5	1.241	26.1
1.067	8.2	1.152	17.6	1.252	27.0
1.075	9.2	1.162	18.6	1.263	28.0
1 083	10.1	1.171	19.5	1.274	28.9
1.091	10.9	1.180	20.5	1.285	29.8
1.100	12.0	1.190	21.4	1.297	30.7
1.108	12.9	1.200	22.4	1.308	31.8
1.116	13.8	1.210	23.3	1.320	32.7
1.125	14.8	1.220	24.2	1.332	33.7
1.134	15.7	1.231	25.1	1.345	34.9

TABLE 3.—SHOWING PERCENTAGE OF NaOH IN AQUEOUS SOLUTIONS CORRESPONDING TO THE SPECIFIC GRAV., TEMP. 15°. (*LUNGE.*)

SPECIFIC GRAVITY.	PERCENTAGE.	SPECIFIC GRAVITY.	PERCENTAGE.	SPECIFIC GRAVITY.	PERCENTAGE.
1.075	6 55	1.162	14.37	1.263	23.67
1.083	7.31	1.171	15.13	1.274	24.81
1.091	8.00	1.180	15.91	1.285	25.80
1.100	8.68	1.190	16.77	1.297	26.83
1.108	9.42	1.200	17.67	1.308	27.80
1.116	10.06	1.210	18.58	1.320	28.83
1.125	10.97	1.220	19.58	1.332	29.93
1.134	11.84	1.231	20.59	1.345	31.22
1.142	12.64	1.241	21.42	1.357	32.47
1.152	13.55	1.252	22.64	1.370	33.69

If, for example, the specific gravity of a solution of potassium hydrate is 1.22, the per cent. of KOH is 24.2; therefore, in 1 cc. weighing 1.22 grm. there is 0.29524 grm. KOH ($\frac{24.2 \times 1.22}{100} = 0.29524$), and in 1000 cc. normal potassium hydrate there being 56 grms. KOH, this quantity is in 189.6 cc. of the solution having the sp. gr. 1.22 ($\frac{56}{0.29524} = 189.6$). But the method of determining the quantity from the specific gravity of a solution is not accurate, otherwise the normal solution could be prepared by diluting 189.6 cc. of the solution with water to 1000 cc. To titrate with normal oxalic acid, the solution is diluted so that the solution be somewhat stronger than that of normal strength. For this purpose 230 cc. of the solution is diluted to 1100 cc. Having mixed well by shaking, the solution is standardized with normal oxalic acid.

Introduce 20 cc. of the solution of potassium hydrate from a burette into a 400 cc. flask, add 100 cc. water and sufficient solution of litmus (the preparation of which is found below) to impart a distinct, but not deep blue, color to the solution. Titrate the solution with normal oxalic acid until, after mixing by agitating the flask, the color of the solution turns purple or red by the addition of 0.1 cc. Repeat the titrations until an agreement is reached, when the solution is diluted so that 20 cc.

will correspond to 20 cc. of the normal oxalic acid. If, for example, 22.2 of the normal acid is required to neutralize 20 cc. of the solution of potassium hydrate to dilute the latter to normal strength, 20 cc would require the addition of 2.2 cc. water and 1000 cc. would require 110 cc. water (20 : 2.2 : : 1000 : x). To prevent a standardized alkaline solution, whether of potassium, sodium or barium hydrate, from absorbing carbonic acid of the air, the bottle containing the solution is provided with a rubber stopper having a hole into which a glass tube is introduced, which is connected with a U-shaped tube containing fragments of soda lime.

NORMAL SULPHURIC ACID (49 grms. H_2SO_4 in 1000 cc. WATER).

As pure concentrated sulphuric acid always contains 1 to 4 per cent. water, the normal acid is not prepared by weighing the concentrated acid on a balance of precision and dissolving in water. Either 60 grms. of the concentrated acid is diluted with water to 1100 cc. or the specific gravity of dilute sulphuric acid is determined with a hydrometer, and the per cent. of H_2SO_4 ascertained by consulting Table 4, and a definite quantity of the acid diluted, so that the solution be somewhat stronger than normal, when it is titrated with normal potassium hydrate. For example, the specific gravity of dilute sulphuric acid is 1.20, the per cent. of H_2SO_4 is 27.1, 1 cc. of the acid would therefore weigh 1.2 grm. and contain 0.3252 grms. H_2SO_4 ($\frac{1.2 \times 27.1}{100} = 0.3252$). As 1100 cc. of normal sulphuric acid contain 53.9 grms. H_2SO_4, so 165.7 cc. of the dilute acid contain about this quantity of H_2SO_4 ($\frac{53.9}{0.3252} = 165.7$), but the solution is prepared stronger for titration, therefore 175 cc. of the dilute acid is diluted with water to 1100 cc. Introduce 20 cc. normal potassium hydrate from a burette into a 400 cc. flask, and having added about 100 cc. water and solution of litmus (the preparation of which is found below), to impart a blue color to the solution, but avoiding an excess titrate with the sulphuric acid—diluted—until, after mixing the solution by agitating the flask, the color turns purple or red by the addition of 0.1 cc. Repeat the process until an agreement is reached, when the acid is diluted to normal strength. If 18.8 cc. of the acid be required to neutralize 20 cc. of the normal potassium hydrate, this quantity of acid would require 1.2 cc. water

to become normal, and 1000 cc. would require 63.8 cc. water (18.8 : 1.2 :: 1000 : x. $x = 63.8$).

TABLE 4.—SHOWING PERCENTAGE OF H_2SO_4 IN DILUTE ACIDS CORRESPONDING TO THE SPECIFIC GRAVITY, TEMP. 15° C. (*KOLB.*)

SPECIFIC GRAVITY.	PERCENTAGE.	SPECIFIC GRAVITY.	PERCENTAGE.	SPECIFIC GRAVITY.	PERCENTAGE.
1.060	8.8	1.142	19 6	1.241	32.2
1.067	9 8	1.152	20.8	1.252	33.4
1.075	10.8	1.162	22.2	1.263	34.7
1.083	11.9	1.171	23.3	1.274	36.0
1.091	13 00	1.180	24 5	1.285	37.4
1.100	14.1	1.190	25.8	1.297	38.8
1.108	15.2	1.200	27 1	1.308	40.2
1.116	16.2	1.210	28.4	1.320	41.6
1.125	17.3	1.220	29.6	1 332	43.0
1.134	18.5	1.231	31.0	1.345	44.4

NORMAL HYDROCHLORIC ACID (36.46 grms. HCl in 1000 cc. WATER).

Mix 190 cc. pure hydrochloric acid, specific gravity 1.12, with about 900 cc. distilled water, or determine the specific gravity of dilute acid, and, from the specific gravity, having ascertained the per cent. of HCl by consulting Table 5, the weight of HCl in 1 cc. of the acid is divided into 36.46; the quotient is about the number of cc. of the acid required for 1000 cc. of the normal acid. For example, the specific gravity is 1.166, the per cent. of HCl is 33. 1 cc. of the acid weighs 1.166 grms. and contains 0.38478 grm. HCl ($\frac{1.166 \times 33}{100} = 0.38478$). As 1 cc. of the dilute acid contains 0.38478 grm. HCl, and in 1000 cc. normal acid there are 36.46 grms. HCl, as many cc. of the dilute acid contains the required quantity of HCl as 0.38478 is contained in 36.46, which is 94.7; therefore, 94.7 cc. of the acid diluted with water to 1000 cc., or 104.2 cc. diluted to 1100 cc., would be about normal strength; but the acid is prepared for standardizing by titrating with normal potassium hydrate somewhat stronger; hence 120 cc. is 8 diluted with water to 1100 cc.

Introduce 20 cc. normal potassium hydrate, page 109, into a 400 cc. flask, add about 100 cc. water and a sufficient quantity of solution of litmus (refer below) to impart a distinct blue color to the fluid, but avoid the addition of any more litmus than necessary. Titrate the solution with the acid (diluted) from a burette until, after agitating the flask, the color of the solution becomes purple or red by the addition of 0.1 cc. Repeat the titrations until an agreement is reached when the acid is diluted to normal strength. If 20 cc. normal potassium hydrate requires 18.1 cc. of the acid, the addition of 1.9 cc. water to this quantity of acid would be required, and 1000 cc. would require dilution to 1104.9 cc. (18.1 : 1.9 : : 1000 : x. x = 104.9).

TABLE 5.—SHOWING PERCENTAGE OF HCL IN DILUTE ACID CORRESPONDING TO SPECIFIC GRAVITY, TEMP. 15°C. (*KOLB.*)

SPECIFIC GRAVITY.	PERCENTAGE.	SPECIFIC GRAVITY.	PERCENTAGE.	SPECIFIC GRAVITY.	PERCENTAGE.
1.067	13.4	1.134	26.6	1.180	35.7
1.075	15.0	1.143	28.4	1.185	36.8
1.083	16.5	1.152	30.2	1.190	37.9
1.091	18.1	1.157	31.2	1.195	39.0
1 100	19.9	1.161	32.0	1.199	39.8
1.108	21.5	1.166	33.0	1.205	41.2
1.116	23.1	1.171	33.9	1.210	42.4
1.125	24.8	1.175	34.7	1.212	42.9

SOLUTION OF LITMUS.

Pulverize 50 grms. litmus and introduce the powder into a flask containing 300 cc. distilled water, warm one or two hours on a water bath, frequently shaking, and decant through a filter. Divide the filtrate into two equal volumes, and by means of a glass rod introduce small quantities of either dilute nitric or sulphuric acid into one portion of the solution until the color becomes purple or red, avoiding the addition of more acid than necessary, when the two volumes are united and 50 cc. strong alcohol added. The solution is preserved by keeping in a dark,

cool place in small bottles filled to the neck, each closed with a cork having a groove cut in one side to permit access of air.

THE BARIUM MIXTURE.

The barium mixture employed in urinary analysis is composed of saturated solutions of barium hydrate and nitrate. It is prepared by dissolving about 100 grms. pure crystallized barium hydrate in 1000 cc. warm water, and when cold, if all the hydrate is dissolved, more is added, and the solution is again well mixed by shaking. The saturated solution of barium nitrate is prepared in the same way, when two volumes of the solution of barium hydrate are mixed with one volume of the solution of barium nitrate. The mixture is kept in well-stoppered bottles.

THE MAGNESIUM MIXTURE.

The magnesia mixture employed in separating phosphoric acid from solution, is prepared by dissolving 56 grms. magnesium chloride in 400 cc. distilled water in a 1000 cc. graduated flask, and adding 70 grms. ammon. chloride, and when solution has taken place, 350 cc. strong ammon. hydrate is added, and the flask is filled with water to the mark.

MILLON'S REAGENT.

Dissolve one part of mercury by weight in one part of concentrated nitric acid by weight. For this purpose a flask may be employed. Usually, the mercury dissolves in a few minutes, when the solution is diluted with an equal volume of water.

CHAPTER IX.

The Quantity of Urine Passed in Twenty-four Hours—Specific Gravity—The Solids—Inorganic Substances—The Coloring Matter—Acidity of the Urine—Urea—Liebig's Method Modified by Pflüger—Estimation of Urea in Diseased Urine by Liebig's Method—Knop's Method Modified by Greene.

THE QUANTITY OF URINE PASSED IN TWENTY-FOUR HOURS.

For the purpose of determining the quantity of urine formed in twenty-four hours, a graduated cylinder of 2000 cc., divided in spaces of 10 cc., is employed, but not having a cylinder so large, one of 500 or 1000 cc. will answer the purpose by emptying and refilling, taking care, however, to drain well before refilling. The result of the quantitative estimation of sugar, albumen, etc., in urine passed, for example, in the morning, is of little value, as the quantity of urine formed during one period varies according to the quantity of fluids taken. The result is of value when it embraces the quantity in urine formed in periods of twenty-four hours. Therefore, the determination of the quantity of urine passed during this period is nearly always the first step in all quantitative estimations of constituents of the urine, whether of normal or diseased urine.

In order that the calculation of the quantity of a constituent of the urine be made simple, round numbers in cc. are taken; for example, if 1560 cc. is the total quantity of urine of twenty-four hours, the number is made 1600. The slight difference does not practically change the result.

SPECIFIC GRAVITY.

There are three methods for determining the specific gravity of the urine, by means of a urinometer, a Mohr-Westphal's balance, and a picnometer. As the results obtained by the use of the urinometer are sufficiently accurate, except in estimating sugar by the fermentation method, it is seldom that the other methods are employed. For the conditions for the employment of the urinometer, refer to Specific Gravity, Chapter VI.

THE SOLIDS OF THE URINE.

By evaporating a definite quantity of urine on a water bath to dryness, and subjecting the residue to the temperature of 100° C. a few hours and weighing the residue, the result is not correct, as some of the urea decomposes during the process of evaporation. The body that escapes is ammonia, and by the absorption and estimation of which accurate results are obtained. However, with less work, and with results equally as accurate, a small quantity of urine, 5 cc., is evaporated in a platinum dish over strong sulphuric acid in vacuo, the air having been removed by means of an air pump. When it is found that by renewing the acid the weight is constant, subtract the weight of the dish from the weight of the dish + contents, the difference is the weight of the solids in 5 cc. urine, and by multiplying by 20 the product is the number of parts in 100 parts of the urine. It has been found that by multiplying the number above 1000 which the urine weighs more than distilled water by 2.33 (Hässer's number) the product expresses the number of parts of solid matter in 1000 parts of the urine; for example, urine having specific gravity of 1020. $20 \times 2.33 = 46.6$ parts solids in 1000 parts urine. The average total quantity of solid matter in the urine of twenty-four hours of an adult is about 60 grms.—between 4 and 5 per cent.

INORGANIC SUBSTANCES.

In a weighed platinum dish evaporate 50 cc. clear or filtered urine on a water bath to dryness, heat the dish carefully over a spirit lamp or Bunsen's burner by keeping the flame, which should not be large, in continued movement, so that all parts of the dish are heated equally. When vapors are no longer given off, remove the lamp, and, when cold, treat the charred mass with some hot distilled water; stir with a glass rod and filter through a small filter paper into a clean flask of about 200 cc. capacity. This process is repeated until the wash water is free of the chlorides, known by forming no cloudiness, when a small quantity is tested with a solution of silver nitrate and some nitric acid. The evaporating dish containing some of the charred mass is then placed on a water bath, and when the contents are dry, it is gradually heated to a dull, red color, when the carbon will gradu-

ally oxidize and a gray ash be left. The filter paper, which may contain some of the insoluble constituents, is dried, ashed in the dish, when the filtrate with wash water in the flask is evaporated in the dish on a water bath to dryness. Care is taken that there is no loss of the filtrate or wash water, and that the flask containing them is rinsed several times with distilled water and the rinsings evaporated in the dish. The dish is then heated carefully to dull redness, placed in a desiccator, and, when cold, it is weighed. The difference in weight between that of the empty dish and that of the dish + contents is the weight of inorganic matter in 50 cc. urine, and by multiplying by two the product is the weight of inorganic matter in 100 cc. urine. If, instead of filtering and evaporating the filtrate, etc., the urine is evaporated to dryness and the residue ashed, part of the chlorides would pass off, while part would fuse and prevent complete oxidation, hence, the result would be incorrect. To avoid these sources of error the method is more lengthy than it otherwise would be.

THE COLORING MATTER.

There is no method for estimating the quantity of urobilin in the urine; besides, it is not the only coloring matter in urine, as shown by the spectroscope.

THE ACIDITY OF THE URINE.

The degree of acidity of the urine is determined by titrating with a standardized solution of potassium or sodium hydrate. The $\frac{1}{10}$ normal is preferable to either the $\frac{1}{5}$ or the normal solution. For the preparation of normal potassium hydrate, refer to page 109. From the normal solution the $\frac{1}{10}$ is prepared by introducing 100 cc. of the solution into a litre (1000 cc.) flask and filling with distilled water to the mark. After mixing well by shaking, the solution is ready for use. In titrating, litmus paper is used as the indicator. The estimation is made by filling a 100 cc. pipette to the mark with the urine, and having introduced it into a beaker glass it is titrated with the $\frac{1}{10}$ normal potassium hydrate from a 25 or 30 cc. burette. The burette, before filling with the solution, should be clean and dry. In titrating, the urine is frequently tested with red litmus paper, and when the paper

changes to purple or blue by the addition of one or two drops of the solution, after stirring well with a glass rod, a sufficient quantity of potassium hydrate has been added, and after standing a short time the meniscus of the fluid in the burette is read. Titrations are repeated until an agreement is reached. The degree of acidity of the urine may be calculated as quantity of oxalic acid. For example, if 100 cc. urine required 20 cc. of the $\frac{1}{10}$ normal solution of potassium hydrate, the quantity of oxalic acid in 20 cc. of the $\frac{1}{10}$ normal solution ($\frac{1}{50}$ of the quantity in 1000 cc.) is 0.126 grm.; hence, the acid salts in 100 cc. of the urine equal in saturating effect 0.126 grm. oxalic acid. Suppose the quantity of urine formed in twenty-four hours is 1600 cc., then $16 \times 0.126 = 2.016$ grms. oxalic acid.

UREA.

Notwithstanding a great many methods for the estimation of urea in the urine have been proposed, Liebig's method, as modified by Pflüger and others, and the method of Knop and Greene, may properly be regarded as the most trustworthy.

LIEBIG'S METHOD AS MODIFIED BY PFLÜGER.

This method is based on the fact that when a solution of mercuric nitrate comes in contact with a solution of urea, a precipitate forms composed of two bodies—

$$2CH_4ON_2,\ Hg(NO_3)_2,\ 3HgO.$$

However, in the reaction there is some nitric acid set free—

$$(2CH_4ON_2 + 4Hg(NO_3)_2 + 3H_2O = (CH_4ON_2)_2Hg(NO_3)_2,\ 3HgO + 6HNO_3),$$

which dissolves more or less of the urea compound and produces changes in its constitution, consequently the acid is nearly neutralized while titrating. When a certain excess of a solution of mercuric nitrate has been added to a solution of urea, and the fluid is brought in contact with a solution of sodium bicarbonate, a yellow precipitate forms; therefore, in titrating, sodium bicarbonate is employed to determine when the urea has combined with the mercury and an excess of the mercuric nitrate is in the solution. It was found that a solution of the mercury salt to form the yellow compound with sodium bicarbonate contains 3.47 milligrms. mercuric oxide in 1cc. From this it is readily seen

that results vary according as dilute or strong solutions of urea are employed, for the excess (3.47 milligrms. HgO in 1 cc.) is the same in either case. In standardizing the mercury solution with a two per cent. solution of urea, the former is diluted so that 20 cc. corresponds to 10 cc. of the solution of urea; therefore, in titrating, the mixture occupies a volume of 30 cc., when the yellow coloration or precipitate takes place by bringing some of the mixture with sodium bicarbonate. The solution or mixture, therefore, contains 104.1 milligrms. HgO, more than is sufficient to combine with the urea ($30 \times 3.47 = 104.1$). Now, suppose that instead of the 2 per cent. solution of urea a 1 per cent. solution be employed, or the 2 per cent. solution be diluted with an equal volume of water and 20 cc. be titrated; in this case the quantity of urea present is the same, but there is 10 cc. more water. When 20 cc. of the mercuric nitrate solution is added, the yellow precipitate will not appear when some of the mixture is brought in contact with sodium bicarbonate, for the reason that 1 cc. of the mixture contains but 2.6 milligrms. HgO instead of the quantity required, 3.47 milligrms. ($\frac{104.1}{40} = 2.6$). To the mixture (occupying the volume of 40 cc. instead of 30 cc.) there is required the addition of 34.7 milligrms. HgO, that there may be present the excess of HgO, required to yield a yellow precipitate with sodium bicarbonate. Without correction, the additional quantity, 34.7 milligrms. HgO, would be accredited to urea that is not present; hence, estimations of urea are too high by employing dilute solutions, or solutions of less than 2 per cent. urea. And in the employment of solutions of greater strength than 2 per cent., the excess of HgO required, in proportion to the amount of urea, is less, consequently the estimations are too low.

The phosphoric acid of the phosphates in the urine combines with the mercury of the nitrate, so that its removal from the urine before making the titration is requisite. The presence of the chlorine of the chlorides in the urine also interferes with the reaction between the mercuric nitrate and urea, as mercuric chloride is formed which does not combine with urea, neither does it yield the yellow precipitate with sodium bicarbonate; consequently, in the presence of the chlorides more mercuric nitrate is required, and its removal from the urine before making the estimation is necessary.

PREPARATION OF SOLUTIONS REQUIRED IN LIEBIG—PFLÜGER'S METHOD.

SOLUTION OF UREA.

Having dried some pure urea by remaining in a watch glass over concentrated sulphuric acid in a desiccator, until by repeatedly weighing there ceases to be any further loss of weight, 2 to 4 grms. is weighed and introduced into a clean, dry flask of 250 or 400 cc. capacity. To the urea add the required amount of distilled water in cubic centimetres, to standardize to the strength of 2 grms. to 100 cc.; therefore, 1 cc. of the solution would contain 0.020 grm. urea, or the solution is 2 per cent. urea.

THE SOLUTION OF MERCURIC NITRATE.

About 87 grms. of the yellow mercuric oxide is put into a porcelain dish, and treated with one volume of nitric acid, sp. gr. 1.20, and one volume of water. Heat on a water bath, and when brown fumes cease to evolve, add portions of the dilute acid until by heating brown fumes are no longer given off. Evaporate to syrupy consistence while it is stirred, until acid vapors cease to evolve. The mercuric nitrate so formed is dissolved in 1100 cc. distilled water, but to avoid the formation of the basic nitrate in preparing the solution, water is added to the salt in small quantities, while the dissolving process is facilitated by stir ring, when the solution is carefully poured into a graduated glass cylinder of 1200 or 1500 cc. capacity, and any undissolved residue in the dish is dissolved in a small quantity of the dilute acid, and the solution is transferred to the cylinder, and, finally, the dish is rinsed with distilled water and the rinsings added to the contents of the cylinder. The solution in the cylinder is diluted by adding water in small quantities, followed by shaking so as to mix well, until the fluid reaches the 1100 cc. mark. Having mixed well by shaking, the solution is ready for standardizing with the solution of urea.

THE SOLUTION OF SODIUM CARBONATE.

Having heated pure sodium carbonate in a dish over a lamp to dryness, 53 grms. is introduced into a litre flask and with distilled. water filled to the mark. In this case the weight of the salt may be approximative.

For the preparation of the barium mixture, refer to Chapter VIII.

SOLUTION OF SILVER NITRATE.

Dissolve pure crystallized silver nitrate in distilled water, so that the strength of the solution will be of 29.075 grms. to one litre of water. Refer to Volhard's method for the estimation of chlorine, Chapter XI.

TO STANDARDIZE THE SOLUTION OF MERCURIC NITRATE.

The solution of mercuric nitrate as prepared above is too strong, and to determine what dilution is required it is titrated with the urea solution. For this purpose, introduce 10 cc. of the urea solution by means of a clean, dry pipette into a small beaker. A 25 or 30 cc. burette is cleaned, dried and filled to the 0 mark with the mercuric nitrate solution, and 19 cc. is introduced without interruption into the beaker with the urea solution, when the mixture is stirred with a glass rod, and titrated with the solution of sodium carbonate from a burette, until the mixture is nearly neutral. 0.2 or 0.3 cc. of the mercury solution is now added, and, after stirring, transfer a drop of the mixture by means of a glass rod to a small quantity of a mixture of sodium bicarbonate and water in a watch glass, placed on a black background, and if the precipitate formed is white add another 0.1 cc. of the mercury solution, and having mixed well by stirring, if a drop of the mixture produces a yellow coloration, or precipitate, with sodium bicarbonate, enough of the mercury solution has been added. If the precipitate is still white, and since neutralizing 0.4 or 0.5 cc. of the mercury solution has been added, the excess of acid is again nearly neutralized, when the mercury solution is added in quantities of 0.1 or 0.2 cc. until a drop of the mixture yields a yellow coloration with sodium bicarbonate. In making the second titration, introduce without interruption within 0.2 cc. of as much of the solution as was required in the first titration; stir well, titrate with the soda solution until nearly neutral, and, having added 0.1 cc. of the mercury solution, test as before, and repeat the process until a yellow coloration is produced with the sodium bicarbonate. If, for example, 19.8 cc. is the agreement, the solution would require the addition 0.2 cc. water to 19.8 cc. in order that 20 cc. corresponds to 10 cc. of the urea solution, but as the volume of the

solution of sodium carbonate diluted the mixture titrated to a greater volume than 30 cc., a correction is necessary, which is made according to Pflüger, by adding the number of cc. of the urea solution and sodium carbonate solution, and from the sum subtracting the number of cc. mercury solution required, and multiplying the remainder by 0.08, the product of which is subtracted from the number of cc. mercury solution employed in the titration. In this example, 13.85 cc. solution of sodium carbonate was required to neutralize the acid—

$$\underset{\text{Urea Sol.}}{10} + \underset{\text{Na}_2\text{CO}_3\text{ Sol.}}{13.85} = 23.85.$$

$$23.85 - 19.8 = 4.05. \quad 4.05 \times 0.08 = 0.324. \quad 19.8 - 0.324 = 19.476.$$

It is seen from this correction that 19.476 cc. of the solution of mercuric nitrate would be required if the volume titrated did not exceed 30 cc. By the addition of 0.52 cc. water to 19.48 cc. the strength of the solution would be correct. To dilute 1 litre 26.6 cc. water is required (19.48 : 0.52 : : 1000 : 26.69). The solution will undergo no change in well-stoppered bottles kept in a dark place.

PREPARATION OF THE URINE FOR TITRATION.

Urine is prepared for titration by separating the phosphoric acid and chlorine. The former is separated first by means of the barium mixture, when the chlorine is separated from the filtrate by adding a definite quantity of the silver solution. To determine the quantity of the silver solution required to separate the chlorine in 10 cc. urine, in which urea is to be estimated, titrations are made, employing Volhard's method, Chapter XI. This having been accomplished, 60 cc. of the urine is introduced into a dry 100 cc. flask from a burette, after which 20 cc. barium mixture is added. Mix well by shaking, and filter through a dry filter paper into a dry beaker or flask. Of the filtrate three-fourths is urine. For titrations, 60 cc. of the filtrate is introduced into a dry flask, and the number of cc. silver nitrate solution required to separate the chlorine in 45 cc. urine is added, as there is this quantity of urine in the 60 cc. Having mixed well by shaking, the solution is filtered through a dry filter paper into a dry flask.

THE TITRATION.

20 cc. of the filtrate from silver chloride is introduced into a small beaker and titrated as above in standardizing the mercuric nitrate solution, but as the quantity of urea in urine is subject to considerable variation, the result of the first titration is approximative, after which within 0.2 or 0.3 cc. of the required quantity of the mercury solution is added without interruption, when the mixture is rendered nearly neutral with the soda solution, and the titrations continued as above.

When the mixture is titrated with the solution of sodium carbonate to neutralize the acid, blue litmus paper is employed to determine when a sufficient quantity has been added; but as carbonic acid is liberated in the reaction, and is partly held in solution, it imparts the acid reaction after all of the nitric acid has combined with the sodium carbonate, for this reason, when the acid reaction of the mixture is slight, the indication is, that enough of the soda solution has been added.

CALCULATION.

If, at the time the titrations are completed (when the formation of the yellow coloration with sodium bicarbonate takes place), the volume of the fluid is more than 30 cc., correction is made as above, in standardizing the mercury solution; in this case the sum of the volumes of urea solution (filtrate) and soda solution exceeds that of the mercury solution. If, on the other hand, the volume of the mercury solution is greater than that of the sum of the urea and soda solutions, the latter is subtracted from the former, the remainder multiplied by 0.08, and the product added to the number of cc. mercury solution. Having made the correction, the volume of urine in the 20 cc. (of the filtrate) titrated is determined. Before the separation of chlorine from the first filtrate three-fourths of its volume was urine, but the volume of silver solution required differs according to the quantity of chlorine present; hence, the number of cc. urine in 20 cc. of the filtrate (quantity titrated) is subject to variation. If, for example, the 45 cc. urine in 60 cc. of the filtrate required 37.4 cc. of the silver nitrate solution to separate the chlorine, then 20 cc. of the filtrate would contain 9.2 cc. urine ($60 + 37.4 = 97.4$ and $\frac{45}{97.4} \times 20 = 9.2$).

As 1 cc. of the mercury solution (correction having been made) corresponds to 0.010 grm. urea, the number of cc. mercury solution employed multiplied by this number would give the number of grms. urea in 9.2 cc. urine, and by the proportion $9.2 : \frac{\text{number grm. urea in 9.2 cc. urine}}{} : : 100 : x$, the per cent. of urea in the urine is determined.

ESTIMATION OF UREA IN DISEASED URINE.

Albumen in urine interferes with the employment of the mercuric nitrate solution in the same way that phosphoric acid does. It is removed by rendering a measured quantity of the urine—500 cc.—distinctly acid with acetic acid, boiling a few minutes in an evaporating dish, and to recover the loss by evaporation transfer to a ½ litre flask; rinse the dish with a small quantity of water and add the rinsings to the urine in the flask. When cold, fill the flask with water to the mark, and having mixed well by shaking, filter through a dry filter paper into a dry flask, and separate the phosphoric acid and chlorine as if the urine were normal. Leucin and tyrosin combine with mercuric nitrate, but ordinarily the quantity of these bodies is so small that the results would differ but little by their absence. In cases of yellow atrophy of the liver, the quantity of leucin and tyrosin in the urine is so increased that this method cannot be employed, as there is no practical method for separating them from the urea. Sugar in the urine does not preclude the employment of this method.

KNOP'S METHOD, MODIFIED BY GREENE.

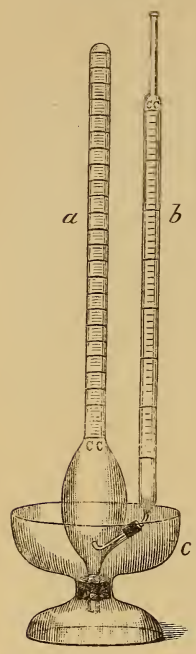
When urea in solution comes in contact with sodium hypobromite dissolved in water with sodium hydrate, nitrogen is set free—

$$CH_4ON_2 + 3NaBrO = CO_2 + 2H_2O + 3NaBr + 2N.$$

The CO_2 is absorbed by the sodium hydrate. The solution is prepared by dissolving 22 grms. sodium hydrate in 250 cc. water, and when cold adding 5 cc. bromine to the alkaline solution. The bromine is measured in a small graduated glass cylinder, and when emptied into the sodium hydrate solution, it is rinsed with the alkaline fluid and rinsings added to the hypobromite solution. The solution of hypobromite of sodium is kept in glass-stoppered bottles in a cool, dark place.

Of the many forms of apparatus for the determination of urea by this method, that of Greene is the least complicated and admits of determinations without consuming much time. It is represented by Fig. 32, and consists of a graduated tube (*a*) of 20 or 30 cc. capacity, and the lower extremity of which is enlarged and provided with a side tube which passes upward at an acute angle. The enlarged portion is drawn out and passes through a cork fitted into the opening in the receiver (*c*). The part (*b*) is a special pipette, having its lower end bent and provided with a cork. The graduated tube and enlarged portion are first completely filled with the hypobromite and bromine solution and placed in the cup (*c*), when three or four cc. urine are introduced into the solution through the side tube by means of the pipette (*b*). The opening or orifice of the pipette is so small that at least a minute is required for the amount of urine to pass into the alkaline solution. In the course of thirty minutes the reaction is complete and nitrogen has collected in the graduated tube. Either the pipette is fixed in place and water added until it stands on an even plane with the fluid in the graduated tube, or, for more accurate results, the pipette is removed and the graduated tube (*a*) is placed in a vessel filled with water, so that the water and fluid in the tube are on an even plane, when the apparatus is secured in place by a holder and allowed to remain until the temperature of the air, water and nitrogen become the same. At the time of reading, the volume of nitrogen, the point at which the latter and the fluid meet, is brought even with the water in the cylinder or vessel. Memoranda of the temperature and barometric pressure at the time of reading are made. The volume of dry nitrogen at zero C. and normal pressure, 760 mm., is determined by the following formula:—

Fig. 32.



$$V \frac{V.\ P-T}{760\ (1 + 0.00366\ t)}.$$

$\acute{V}$ = Volume of nitrogen in cubic centimetres.
V = Number of cubic centimetres nitrogen read.
P = Barometer reading.
T = Vapor tension in millimetres.
t = Thermometer reading.

Vapor tension at ordinary temperatures is here given in millimetres:—

10° C.	9.126	15° C.	12.677	20° C.	17.396
11 "	9.751	16 "	13.519	21 "	18.505
12 "	10.421	17 "	14.409	22 "	19.675
13 "	11.130	18 "	15.351	23 "	20.909
14 "	11.882	19 "	16.345	24 "	22.211

In one gramme of pure urea there is 371.4 cc. dry nitrogen at normal pressure and temperature; but it has been found by this method that 354.3 cc. nitrogen is liberated from one gramme urea. This number is therefore employed in calculating the amount of urea from the volume of nitrogen, as shown by the equation, $354.3 : 1 :: \frac{\text{number of cc.}}{\text{nitrogen.}} : x$, when calculation is made of the quantity of urea in 100 cc., or in the total quantity of urine formed in twenty-four hours.

CHAPTER X.

Determination of the Total Quantity of Nitrogen in Urine—Dumas' Method—Varrentrapp and Will's Method—Kjeldahl's Method—Remarks on the Estimation of the Quantity of Urea and Nitrogen in Urine—Uric Acid—Salkowski's Method, modified by E. Ludwig—Kreatinin—Neubauer's Method, modified by Salkowski—Oxalic Acid—Neubauer's Method, modified by Fürbringer.

DETERMINATION OF THE TOTAL QUANTITY OF NITROGEN IN URINE.

Of several methods for estimating the quantity of nitrogen in urine, three are here given—Dumas', Varrentrapp and Will's, and Kjeldahl's. By the method of Dumas, nitrogen is collected and measured, and by those of Varrentrapp and Will, and Kjeldahl, ammonia is formed and the quantity estimated by volumetric or gravimetric methods.

DUMAS' METHOD.

This method is based on the fact that organic bodies containing nitrogen, when heated with copper oxide, are oxidized with the liberation of nitrogen, and any oxygen compounds of nitrogen that may be formed are decomposed by metallic copper heated to redness. The weight of the nitrogen liberated is determined from the volume it would occupy at zero C. and 760 mm. barometric pressure. The nitrogen is collected in a 200 cc. graduated glass tube or cylinder with ground margins. The combustion tube employed is 70 to 80 cm. long with one end sealed by heating in the flame of a blast lamp. About 15 cm. of the sealed end is filled with dry sodium bicarbonate,* followed by about 10 cm. with finely granulated copper oxide, when a porcelain boat, containing the mixture of the solids of the urine and finely granulated copper oxide, is introduced. Formerly, urine, 5 cc., having been acidified with dilute sulphuric acid, was evaporated at 100° C. to near dryness, and when cold mixed in a porcelain boat with finely granulated copper oxide by employing a piece of wire, when the mixture was covered with copper

* Pure dry manganese carbonate may be employed instead of sodium bicarbonate. It has the advantage of not liberating water by heating.

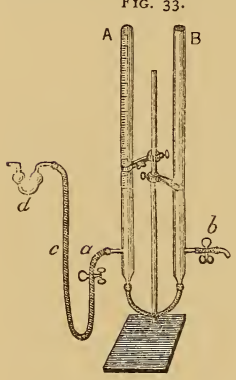
oxide and the boat introduced into the tube; but the process has been shortened (Horbaczewski) by introducing 5 cc. urine into the porcelain boat half full of pulverized CuO, after which the boat is nearly filled with dry granulated CuO, when it is introduced into the combustion tube. Following the boat, finely granulated copper oxide is introduced, about 10 cm., when a roll of copper foil, 15 cm., is placed in the tube, and, finally, some more granulated CuO is introduced. The empty space of 5 cm. from the CuO to the end of the tube is cleansed with some porous paper, and by means of a tightly-fitting cork or rubber stopper connection is made with the delivery tube. To prevent the return of the water that may be formed in the open space at the end of the tube, the furnace is inclined somewhat. The graduated cylinder is filled two-thirds with mercury, air bubbles that may adhere to the glass are removed with a stirring rod, when it is filled to the margin with a strong solution of potassium hydrate. Slide a piece of ground glass over the ground surfaces of the cylinder, and if air bubbles are excluded invert the cylinder and place its open end, secured by the piece of ground glass, under the surface of mercury of the bath and retain the cylinder in place by a holder. About one-half of the sodium bicarbonate or manganese carbonate (6 cm. of the sealed end of the tube) is heated to redness. By decomposition of the $NaHCO^3$ carbonic acid is evolved, which drives the air out of the tube.

$$2NaHCO_3 = H_2O + Na_2CO_3 + CO_2.$$

To determine when this has taken place, collect some of the gas in a test tube filled with a solution of potassium hydrate over mercury from the delivery tube, and if the air has been driven out of the combustion and delivery tubes, and no air has been admitted from without, the gas will be completely absorbed. When this has taken place, the end of the delivery tube is placed under the mouth of the graduated cylinder, and the roll of copper foil in the combustion tube and the CuO adjacent thereto are heated to redness, and the application of heat is continued toward the boat. The lamps of the furnace under the boat are not lighted until the water of the urine is evaporated by the heat of the CuO a few centimetres from the boat. This having taken

place, apply heat gradually to the part of the tube in which the boat is until heated to redness. When complete combustion has taken place, known by no more nitrogen entering the graduated cylinder, the remainder of the sodium bicarbonate (or manganese carbonate) is heated gradually, that carbonic acid gas evolved drives out all of the nitrogen from the combustion and delivery tubes. The process having been completed and the delivery tube removed, the gas is turned off from the lamps, and the graduated tube having remained in position about thirty minutes, is transferred to a glass vessel, a large beaker glass for example, filled with water. This is brought about by placing a small evaporating dish under the mouth of the tube in the mercury, and while it is retained in the mercury in the evaporating dish it is removed to the vessel containing water. By removing the dish under the surface of the water, the mercury will sink to the bottom of the vessel and water will take its place. When the nitrogen, air and water are of the same temperature, the surface of the fluid in the cylinder is brought even with that of the surrounding water by lowering or raising the tube in the holder when the volume of nitrogen is read ; at the same time readings of the thermometer and barometer are made. Instead of employing a simple graduated cylinder for collecting and measuring the nitrogen, Zulkowsky's azotometer (Fig. 33) may be employed.

Fig. 33.



This apparatus comprises two tubes, A and B, each of which is 58 cm. long and 18 mm. diameter. Tube A is graduated, its upper end sealed, and by means of a piece of glass tubing welded in tube A, a few centimetres from the lower extremity, the rubber tube *c* and the small U-shaped tube *d*, it is connected with the combustion tube. Both tubes are filled with a strong solution of potassium hydrate, by first filling the tube B, and then by inclining the apparatus the solution will run into tube A and the air pass out. This process is repeated until both tubes are filled, when some of the solution is allowed to escape through the tube secured by the pinch-cock *b*. If, when the first portion of the sodium bicarbonate is being

decomposed and the air driven out of the combustion tube, connection having been made with tube A, it is found that all of the gas which enters the tube is absorbed, the air has been expelled. If, however, air collects in the tube, it is removed by inclining the apparatus as in filling with the solution of sodium hydrate. During the combustion the pinch-cock *a* is removed, and that some unabsorbed carbonic acid may not cause an overflow of the tube B, some of the fluid is drawn off through the tube secured by the pinch-cock *b*. When the combustion has been completed, and all of the nitrogen driven out of the combustion and connecting tubes into tube A, the apparatus is disconnected, the tube again secured by the pinch-cock, and a strong solution of potassium hydrate is introduced into tube B, and, after standing some time, a thermometer is introduced into the solution in this tube, to ascertain the temperature. The pinch-cock *b* is opened to let sufficient sodium hydrate solution escape, to bring the fluid in both tubes even when reading of the barometer is made.

CALCULATION.

For the formula for the reduction of the volume of nitrogen, read to that of zero and 760 mm. pressure, refer to calculation of the quantity of urea from nitrogen, Knop and Greene's method, page 125; and having determined the volume of nitrogen at normal temperature and pressure, multiply the volume (number of cc.) by 0.001256, the product of which is the number of grammes nitrogen in 5 cc. urine.

VARRENTRAPP AND WILL'S METHOD.

When nitrogenous compounds of the urine are mixed with soda-lime (a compound of sodium and calcium hydrates) and heated to redness, the nitrogen combines with hydrogen of the hydrates, forming ammonia, NH_3. The ammonia gas evolved is conducted through dilute sulphuric acid (normal sulphuric acid), by which it is absorbed, forming $(NH_4)_2\,SO_4$. The combustion having been completed, the acid remaining uncombined is determined by titrating with a solution of potassium hydrate of known strength. For the preparation of normal sulphuric acid, refer to page 112, and to prepare normal potassium hydrate, page 109. The one-fifth normal potassium hydrate employed in

titrating is prepared from the normal solution by introducing 100 cc. of the latter into a 500 cc. graduated flask and filling with distilled water to the mark. Soda-lime may be procured of dealers in chemical reagents. Unless it is free of nitrates or nitrites, it will be a source of error. To test for these impurities, mix the soda-lime with some pure cane sugar and heat in a tube sealed at one end to redness; ammonia is liberated, known by changing moistened turmeric paper dark red if nitrogen compounds are present. Soda-lime is dried before using by warming in a porcelain dish.

The combustion tube employed is about 40 cm. long and 12 mm. in diameter, one end of which is drawn out to a fine point, sealed, and where the contraction begins it is bent so that the sealed extremity is several centimetres above the furnace. The sharp edges of the open end of the tube are rounded by heating in the flame of a blast lamp, care being taken not to heat sufficient to cause any irregularity in the opening. The absorption apparatus (Varrentrapp and Will's) comprises three bulbs communicating with each other by tubular openings. One of the bulbs is pear-shaped and is connected with the combustion tube by a glass tube passing through a cork fitting in the former. In filling the combustion tube, a tuft of asbestos is placed in the tube where the contraction begins, in front of which a small quantity of soda-lime—4 to 5 cm.—is placed, when a mixture of the solids of the urine and soda-lime is introduced. For this purpose, 5 cc. urine and an equal volume saturated solution of oxalic acid are mixed in a small evaporating dish with a sufficient quantity powdered calcium sulphate (gypsum) to form a pasty mass, when it is dried at 100° C. Instead of a dish a Hofmeister's capsule may be employed with advantage. This capsule is of very thin glass, so that it may be pulverized with its contents. In either case the dried mass is intimately mixed with some soda-lime in a mortar (avoiding heavy pressure with the pestle) and introduced into the combustion tube. The part of the mixture adhering to the mortar is removed by triturating small quantities of soda-lime, and adding to the mixture in the combustion tube. The part of the tube occupied by the mixture should be about 18 cm. in length. Finally, the tube is filled to 5 cm. of the end with soda-lime, and any particles of soda-lime in the open space are removed with

some soft paper, when a tuft of clean asbestos is fitted in the open space. By gently tapping the upper part of the tube space is formed for the passage of gases or vapors evolved. Into a 200 cc. beaker introduce. 10 cc. normal sulphuric acid from a burette, and having placed the end of the tube of Varrentrapp and Will's absorption apparatus, connected with the round-like bulb, draw the acid into the bulbs by suction. The acid not being in sufficient quantity to fill the third or pear-shaped bulb half full when the apparatus is placed in the horizontal position, some water is introduced into the beaker with the acid yet remaining, and it is drawn into the bulbs as before. The beaker is then covered with a glass plate or large watch glass and placed one side, as it contains some of the 10 cc. normal acid. By means of a well-fitting rubber or cork stopper the absorption apparatus is connected with the combustion tube. To determine if the apparatus is air tight, warm the air in the pear-shaped bulb by holding a glowing coal or piece of hot iron near the surface of the glass, and by the expansion of the heated air a few bubbles will pass through the acid, and if the apparatus is air tight the acid in the pear-shaped bulb will remain at a higher plane than before. The anterior part of the tube containing the soda-lime is heated to redness, but care is taken not to heat the mixture until the soda-lime in front has reached a red heat; at the same time gas in one or two lamps at the bend of the tube is turned on and ignited, so that the soda-lime contiguous to the tuft of asbestos be heated to redness and the drawn-out portion of the tube reach a degree of temperature sufficient to prevent the condensation of aqueous vapor. The application of heat is now continued slowly toward the sealed end of the tube until the tube is heated to redness from the bend to the tuft of asbestos in its anterior part. At no time during the process should the temperature of the soda-lime in the anterior part of the tube be reduced, neither should the heat be so intense as to incur the risk of decomposing the ammonia. The combustion is complete if, while the contents of the tube are heated to redness, the fluid in the bulbs is drawn toward the furnace. When this takes place, the gas is turned off and air is drawn through the apparatus to cause the absorption of the ammonia remaining in the tube. This is brought about by connecting an aspirator with the free extremity of the bulbs, and the sealed end

of the tube is broken by means of a pair of tongs. When about one litre water in the aspirator has been displaced by air (requiring about twenty-five minutes), the process is complete, and the quantity of acid in the bulbs is determined by titrating with the one-fifth normal potassium hydrate. For this purpose, the fluid is emptied into the beaker, which yet contains some of the 10 cc. normal acid, and after rinsing the bulbs several times with distilled water and adding the rinsings to the acid in the beaker, a sufficient quantity of solution of litmus (refer to page 114) is added to impart a distinct red color to the solution, when it is titrated with the one-fifth normal potassium hydrate from a burette until, by the addition of 0.1 cc., the color changes purple or blue.

CALCULATION.

In 1 cc. normal ammonia there is 0.017 grm. NH_3 or 0.014 grm. nitrogen. 1 cc. normal acid will neutralize 1 cc. normal ammonia; therefore, by multiplying the number of cc. normal acid neutralized by 0.017, the product is the quantity of NH_3 in grammes, or, by multiplying by 0.014, the number of grammes nitrogen is determined.

Example: 31 cc. of one-fifth normal potassium hydrate was required to neutralize the acid instead of 50 cc., as would be the case in the absence of ammonia. 31 cc. one-fifth normal solution is equal to 6.2 cc. of the normal ($\frac{31}{5} = 6.2$), and as 10 cc. of the normal sulphuric acid was employed, 3.8 cc. was neutralized by the ammonia formed from 5 cc. urine ($10 - 6.2 = 3.8$), and as 3.8 cc normal ammonia would neutralize 3.8 cc. normal sulphuric acid, there is in 3.8 cc. normal ammonia the quantity of nitrogen found in 5 cc. urine, which is 0.0532 grm. ($3.8 \times 0.014 = 0.0532$), and in 100 cc. urine there is 1.064 grm. nitrogen ($0.0532 \times 20 = 1.064$).

KJELDAHL'S METHOD.

This method is based on the fact that an organic body containing nitrogen, when heated with either fuming sulphuric acid or a mixture of fuming sulphuric acid and concentrated sulphuric acid, sp. gr. 1.84, the nitrogen passes into the compound ammonium sulphate $(NH_4)_2SO_4$. By distilling a solution of the compound with an excess of sodium hydrate, ammonia is liberated, and in the receiver it is combined with a definite quantity

of sulphuric acid. When the process is complete, the amount of ammonia formed is determined by titrating the acid with one-fifth normal potassium hydrate. The method here given is nearly the same as that of Kjeldahl, as it is found accurate in estimating nitrogen in urine, blood and fæces; but for the determination of nitrogen in some other bodies it has been modified.

SOLUTIONS REQUIRED.

1. Fuming sulphuric acid.
2. Normal sulphuric acid.
3. One-fifth normal potassium hydrate.
4. A solution of sodium hydrate, specific gravity 1.3.
5. A solution of litmus.

For the preparation of normal sulphuric acid, refer to page 112.

The one-fifth normal potassium hydrate is prepared by introducing 100 cc. of the normal solution (for the preparation of which refer to page 109) into a 500 cc. graduated flask and filling with distilled water to the mark. To prepare a solution of sodium hydrate, sp. gr. 1.3, dissolve 320 grms. sodium hydrate in about 500 cc. water, and, when cold, the solution is introduced into a 1000 cc. graduated flask and filled with water to the mark. As a high temperature is produced by dissolving sodium hydrate in water, place the flask or bottle containing the water and hydrate in cold water, and mix well by shaking, to prevent the parts of the glass in contact with the sodium hydrate from becoming so hot as to endanger the vessel. For the preparation of a solution of litmus, refer to page 114.

APPARATUS REQUIRED.

A 200 cc. round-bottom Bohemian flask.

A 750 cc. Erlenmeyer flat-bottom flask, a condenser, and a 400 cc. flask as a receiver, a safety tube, besides a 50 cc. burette, etc.

THE ESTIMATION.

Introduce 5 cc. urine from a burette into a 200 cc. round-bottom flask, and add 10 cc. fuming sulphuric acid.*

To prevent loss while the fluid is heated, introduce (according

* The fuming acid is conveniently measured in a small graduated glass cylinder or a test tube graduated for 10 cc. by a mark made with a sharp file.

to Arnold) into the mouth of the flask a test tube which fits loosely into its neck, having been enlarged in its upper third by heating in the flame of a blast lamp and blowing out. If the enlargement of the test tube is near or in its middle third, remove the upper part of the tube with a file. Place the flask on wire gauze secured at an angle of 45°, and heat the fluid with a gas or spirit lamp. Continue the application of heat until the fluid becomes light yellow in color. This usually takes place in one to one and one-half hours. The fluid should be kept in constant ebullition. After cooling, place the flask in cold water, as heat is generated by diluting, and add water in small quantities, mixing well by shaking gently after each addition, and when the dilution has reached about 100 cc. the fluid is introduced into the 750 cc. Erlenmeyer flask. Rinse several times with water, and add the rinsings to the fluid. The quantity of fluid in the Erlenmeyer flask should not exceed 200 cc. Into the 400 cc. flask to receive the distillate, introduce 10 cc. normal sulphuric acid from a burette. The condensing tube of the cooler should be somewhat long, and the end to enter the receiver bent, that its orifice may be brought as near the acid as possible without coming in contact. As the fluid is dense when the solution of sodium hydrate is introduced, it is liable to bump during the distillation, to prevent which small fragments of zinc are introduced into the flask. By the action of NaOH on zinc, hydrogen is evolved, which prevents the bumping; but it was found (Pfeiffer and Lehmann) that with the hydrogen and aqueous vapor a small quantity of sodium hydrate is carried over, hence a safety tube is introduced between the Erlenmeyer flask and condenser. This is made by drawing out the end of a combustion tube, 20 cm. long and 18 mm. internal diameter, in the flame of a blast lamp to 8 or 10 mm. which passes through a hole in the cork of the Erlenmeyer flask.* The upper end of the tube is connected with the cooler by means of a cork, through which passes a bent glass tube. To the fluid to be distilled add 60 cc. of the solution of sodium hydrate (sp. gr. 1.30) and three fragments of zinc as nearly spherical as possible, the weight of which not to exceed 0.5 grm. The sodium

* It was found in this laboratory, that by a safety tube of the dimensions here given, the purpose is accomplished as well as with others more complex in construction which have been recommended.

hydrate and zinc having been introduced, connection with the condenser is made at once to prevent loss of ammonia. Distill slowly until the ammonia separates from the fluid and is carried into the receiver and absorbed by the sulphuric acid. This is usually accomplished by distilling thirty minutes. To determine with greater accuracy if all the ammonia is distilled, place a small piece of red litmus paper at the orifice of the condensing tube, and if ammonia is still passing over, the red litmus paper turns blue. The quantity of ammonia absorbed by the 10 cc. normal sulphuric acid is determined by titrating with the one-fifth normal potassium hydrate. For this purpose, the solution of litmus is added to the fluid in quantity sufficient to impart a distinct red color, when the solution is titrated with the one-fifth normal potassium hydrate from a 50 cc. burette, until by the addition of 0.1 cc., after shaking, the solution turns purple or blue.

CALCULATION.

For the determination of the quantity of nitrogen from the quantity of acid neutralized in the titration, refer to calculation employed in Varrentrapp and Will's method, page 131.

REMARKS ON THE ESTIMATION OF UREA AND NITROGEN.

There is no method by which the exact amount of urea in urine is estimated, although the variation in results with some of the methods is not great. The difficulty is, other bodies in the urine either combine with, or are decomposed by, the reagent employed, according to the method. There is perhaps no method in quantitative analysis which has been more closely studied, and been the subject of discussion to such an extent, as Liebig's method, as variously modified, and although complicated, yet for clinical purposes it is satisfactory. To determine the amount of waste of the tissues, as in fever, exhaustive labor, or the waste from the transformation of nitrogenous elements of food in the blood, the most reliable methods are those by which all of the nitrogen in the urine is determined. For this purpose, the only objection, so far as the results are concerned, to Dumas' method is the great difficulty, if not impossibility, of separating all of the air from copper oxide (Kreusler), and in very few cases, for example, the presence of nitro-substitution compounds in the

urine, is there any objection to the method of Varrentrapp and Will. The results of Kjeldahl's method obtained in this laboratory are entirely satisfactory, besides, the time saved by employing this method is very great. Having the solutions prepared and the apparatus at hand, a half dozen estimations may be made during the time required to complete one estimation with the furnace.

URIC ACID.

SALKOWSKI'S METHOD, MODIFIED BY E. LUDWIG.

This method of determining the quantity of uric acid in urine is based on the facts that silver urate is insoluble in solutions rendered strongly alkaline with ammon. hydrate, and that when suspended in water it is decomposed with sodium sulphide, forming silver sulphide and sodium urate. The phosphates of calcium and magnesium are separated with the silver sulphide by filtering.

For the employment of this method, three solutions are required: A solution of silver nitrate, an alkaline solution—ammonia solution—of the double chloride of magnesium and ammonium or "magnesia mixture," and a solution of sodium sulphide.

Dissolve 26 grms. crystallized silver nitrate in 200 or 300 cc. distilled water in a 1000 cc. graduated flask, add an excess of ammon. hydrate and with water fill to the mark. Mix well by shaking.

For the preparation of magnesia mixture, refer to page 115.

Dissolve 10 grms. sodium hydrate in 1000 cc. water. Mix well by shaking, and treat 500 cc. of the solution with sulphureted hydrogen gas to saturation, when the two solutions are mixed, forming sodium sulphide.

$$NaOH + H_2S = NaHS + H_2O \text{ and } NaHS + NaOH = Na_2S + H_2O.$$

Having prepared the three solutions, introduce 25 cc. of the silver solution and an equal volume of the magnesia mixture into a flask, add ammon. hydrate until the precipitate AgCl dissolves. This mixture is added to 200 cc. filtered urine and mixed well with a glass rod. After the lapse of an hour filter through a filter secured by a platinum cone, and to facilitate the process connection is made with a filter pump or an aspirator bottle. Wash

the precipitate three times with water containing some ammon. hydrate. Transfer the precipitate to the beaker in which the precipitation took place by means of a stream of water from a wash bottle, taking care, however, not to injure the filter. To the precipitate, suspended in the water, the sodium sulphide solution is added in sufficient quantity to precipitate the silver as sulphide, when the mixture is heated until the boiling point is reached, while it is constantly stirred. After cooling, filtert hrough the filter employed before into an evaporating dish, and wash with hot water until a sodium hydrate solution of lead acetate ceases to produce a precipitate or dark coloration with the wash water. Render the filtrate with wash water in the dish faintly yet distinctly acid with dilute hydrochloric acid, and concentrate by evaporating on a water bath to the volume of about 15 cc. Let stand three hours in a cool place, when the uric acid will separate. Filter through a small, weighed filter paper, having been dried at 110° C. The filtrate is employed in connection with a stirring rod, over the end of which is placed a short piece of rubber tube, in bringing all of the uric acid on the paper, when it is washed several times with water and dried in the funnel, but at a temperature not exceeding 110° C. The funnel and filter having cooled, the uric acid is washed with about 10 cc. carbon bisulphide in three portions, after which wash with a small quantity of ether and dry at 110°, and after cooling in a desiccator, weigh. The process of heating, cooling and weighing is repeated until the weight becomes constant. The difference between the weight of the filter and that of the filter + precipitate is the weight of uric acid in 200 cc. urine, or twice the per cent. of uric acid in the urine, not taking the specific gravity of the urine into consideration.

KREATININ.

NEUBAUER'S METHOD, MODIFIED BY SALKOWSKI.

After the separation of phosphoric acid the urine is evaporated to dryness, an alcohol solution of constituents of the residue soluble in alcohol prepared, and the kreatinin precipitated with an alcohol solution of zinc chloride. To prepare the alcohol solution of zinc chloride, the syrupy chloride is treated with strong alcohol until the specific gravity of the solution becomes 1.2, when it is filtered. Introduce 240 cc. of the urine into a graduated 300 cc.

flask, render slightly alkaline with milk of lime, and treat with a solution of calcium chloride until a precipitate ceases to form. Fill the flask with water to the mark and mix well by shaking. After standing fifteen minutes, filter through a dry filter paper. The filtrate should be alkaline in reaction; but if decidedly so, neutralize with hydrochloric acid, but not until 250 cc. of the filtrate is introduced into an evaporating dish. Evaporate on a water bath to about 20 cc., and when cold add an equal volume of absolute alcohol. Mix well by stirring with a glass rod, and introduce the fluid into a graduated 100 cc. flask. Rinse the dish several times with absolute alcohol and add the rinsings to the fluid in the flask, and fill the flask with absolute alcohol to the mark. Mix well by shaking after having fitted the stopper in place. Having stood twenty-four hours, filter through a dry filter paper and introduce 80 cc. of the filtrate into a beaker glass. To the alcohol solution add about 1 cc. of the alcohol solution of zinc chloride; mix well by stirring with a glass rod several minutes; finally cover with a glass plate or large watch glass and let stand in a cool place two or three days. The solution is then filtered through a small, weighed filter paper which was dried at 100° C. The filtrate is employed in collecting and bringing the precipitate on the filter by means of a stirring rod, the end of which is provided with a small piece of rubber tubing. Wash the precipitate with small quantities of strong alcohol until the alcohol filtrate is free of chlorine, known by testing with a solution of silver nitrate and a few drops of dilute nitric acid. When washed, the filter paper with precipitate is dried and weighed as before.

CALCULATION.

The difference in weight between the filter with watch glasses (between which the filter was weighed) and the filter + precipitate with watch glasses, is the weight of kreatinin zinc chloride. 240 cc. urine is diluted to 300 cc., and when filtered, 250 cc. of the filtrate is taken, which corresponds to 200 cc. urine ($\frac{250}{300} \times 240 = 200$). Of the 100 cc. alcohol solution, 80 cc. is taken, and as the 100 cc. solution contains the kreatinin of 200 cc. urine, 80 cc. contains the kreatinin of eight-tenths as much urine, which is 160 cc., and five-eighths of this quantity of kreatinin corresponds to 100 cc. urine ($\frac{100}{160} = \frac{5}{8}$); therefore, by mul-

tiplying the weight of kreatinin zinc chloride, weighed, by five-eighths, the product of which is the weight of the compound from 100 cc. urine, and as 62.44 per cent. of the zinc compound is kreatinin, by multiplying by 0.6244, the product is the weight of kreatinin in 100 cc. urine.

OXALIC ACID.

NEUBAUER'S METHOD, MODIFIED BY FÜRBRINGER.

The quantity of urine passed in twenty-four hours is measured and treated with a few cc. oil of thyme, to prevent bacterial growth. Treat the urine with ammon. hydrate until, after stirring, the odor of ammonia is perceptible. Add a solution of calcium chloride until a precipitate ceases to form, and with acetic acid render distinctly acid, avoiding a great excess. The phosphates of calcium and magnesium dissolve in acetic acid, while calcium oxalate precipitates with more or less uric acid. Let stand twenty-four hours, filter through a small filter paper, collect and transfer the precipitate to the paper with a glass rod provided with a small piece of rubber tubing on one end. Wash with water until the wash water is free of chlorides, known by testing with a solution of silver nitrate and a few drops of nitric acid. When washed, transfer the filter, with precipitate, to a small beaker and treat with dilute hydrochloric acid and water, avoiding a great excess of the former; warm on a water bath, and stir with a glass rod so the acid will come in contact with every part of the precipitate. The calcium oxalate will dissolve in the acid, while any uric acid present will remain undissolved. Filter, through a small filter paper, into a beaker of 250 or 300 cc. capacity, wash with water and determine when washed, as before. Evaporate the filtrate with wash water to about 200 cc., transfer from the dish to a beaker, rinse the dish with water and add rinsings to the fluid in the beaker when the fluid is rendered alkaline with ammon. hydrate, known by turning turmeric paper dark red after the fluid is well mixed by stirring. Having stood well protected from dust twenty-four hours, filter through a small filter paper free of ash, wash with water until the wash water is free of chlorine, known by producing no turbidity when tested with a solution of silver nitrate with a few drops of nitric acid. Dry the filter with pre-

cipitate and ash in a platinum crucible, and by the heat of a good blast lamp reduce the oxalate to the oxide (CaO), let cool in a desiccator and weigh. Repeat the process of heating and weighing until the weight becomes constant. To find the weight of the oxalic acid; $C_2H_2O_4$, multiply the weight of the oxide of calcium, CaO, by 1.6071.

CHAPTER XI.

Phosphoric Acid of the Phosphates and of Glycerin-Phosphoric Acid—Neubauer and Zuelzer's Method—Phosphoric Acid of the Phosphates—Total Quantity of Phosphoric Acid—Sulphur Compounds—Sulphuric Acid or Sulphur of Sulphates and Ester Compounds—The Gravimetric Method—The Volumetric Method (Wildenstein, Brüggelmann and Neubauer)—Sulphuric Acid of the Ester Compounds—Sulphur not in Sulphates or Ester Compounds—Method of Estimating Total Quantity of Sulphur in Urine.—Chlorine, Volhardt's Method, Modified by Salkowski—Neubauer's Method—Remarks on the Methods of Estimating Chlorine in Urine—Potassium and Sodium—Ammonia, Schlösing and Neubauer's Method—Calcium and Magnesium Oxides—Calcium Oxide—The Gravimetric Method—The Volumetric Method (Neubauer)—Magnesium Oxide—The Gravimetric Method—The Volumetric Method (Neubauer).

PHOSPHORIC ACID OF THE PHOSPHATES AND OF GLYCERIN-PHOSPHORIC ACID.

NEUBAUER AND ZUELZER'S METHOD.

Phosphorus in the urine is in the form of simple phosphates, and in an organic body glycerin-phosphoric acid (refer to page 34). The latter body is decomposed by heating with dilute nitric acid, with the liberation of phosphoric acid, which is precipitated by ammon. hydrate and magnesia mixture. Without this preliminary treatment the glycerin-phosphoric acid would remain in solution; hence, by means of the magnesia mixture, the two phosphorus compounds are separated. The magnesium ammon. phosphate, $MgNH_4PO_4$, precipitated by the magnesia mixture, whether of the phosphates or of the total quantity of phosphorus in the urine, is filtered, washed and transferred to a beaker, dissolved in acetic acid and titrated with a solution of uranium acetate of known strength. Formerly, the urine was titrated directly with the uranium solution—free acetic acid present—but by the researches of Zuelzer the results were found too high.

SOLUTIONS REQUIRED.

1. Magnesium mixture.
2. Solution of sodium phosphate.
3. Solution of uranium acetate.
4. Solution of sodium acetate.

PREPARATION OF SOLUTIONS.

For the preparation of magnesium mixture, refer to page 115.

SOLUTION OF SODIUM PHOSPHATE.

From a solution of sodium phosphate of known strength a solution of uranium acetate is standardized. The solution for this purpose contains 10.0845 grms. sodium phosphate, Na_2HPO_4, $12H_2O$, in 1000 cc. water or 0.2 grm. P_2O_5 in 100 cc water. The pure phosphate, with water of crystallization, may be weighed, put into a dry flask or bottle, and, by calculation, the required amount of water be added. For example, if 6.2831 grms. of the salt is weighed on a balance of precision, the quantity of water in which to dissolve the salt is calculated by the equation 10.0845 : 1000 : : 6.2831 : x (x = 623.4 cc.). With greater accuracy, about 7 grms. pure sodium phosphate is dissolved in 600 cc. water, and when dissolved and well mixed by shaking, 50 cc. of the solution is evaporated to dryness in a weighed platinum dish, and with the cover on the dish it is gradually heated until the bottom of the dish becomes dull red in color. Having cooled in a desiccator, it is weighed; the increase in weight is due to sodium pyrophosphate, $Na_4P_2O_7$, formed by the decomposition of the ordinary phosphate by heat. The quantity of the sodium pyrophosphate in 50 cc. of the solution corresponding to the quantity of sodium phosphate required is 0.1873 grm., and 500 cc. of the solution is diluted accordingly. If, for example, 50 cc. of the solution yields 0.1950 grm. of the pyrophosphate, the quantity of water with which to dilute 50 cc. of the solution is determined by the equation 0.1873 : 50 : : 0.1950 : x. x = 52.05. If 50 cc. requires 2.05 cc. water, 500 cc. would require 20.5 cc. (10 × 2.05 = 20.5).

SOLUTION OF URANIUM ACETATE.

About 44 grms. uranium acetate is dissolved in 1100 cc. water, or 35 grms. of the oxide of uranium is dissolved in acetic acid and the solution diluted with water to 1100 cc. This solution is standardized with the sodium phosphate solution, as directed below.

SOLUTION OF SODIUM ACETATE.

Dissolve 100 grms. pure sodium acetate in water, introduce the solution into a 1000 cc. graduated flask, and fill with water to the mark.

SOLUTION OF POTASSIUM FERROCYANIDE.

10 grms. of potassium ferrocyanide is dissolved in 100 cc. water.

STANDARDIZING THE SOLUTION OF URANIUM ACETATE.

When a solution of uranium acetate is brought in contact with a solution of sodium phosphate a light yellow precipitate forms—

$$2UOC_2H_3O_2 + Na_2HPO_4 = (UO)_2HPO_4 + 2NaC_2H_3O_2.$$

If the uranium solution be added to the phosphate, and there be mixed with the latter some potassium ferrocyanide and sodium acetate in solution, uranium ferrocyanide (a dark-colored precipitate) will not form unless the phosphoric acid has combined with uranium, and there is in the solution an excess of uran. acetate. In titrating, therefore, potassium ferrocyanide is used as the indicator. In titrating solutions in which there is an acid beside acetic acid, the sodium acetate is added that the sodium of the acetate combine with the acid (or the acid of an acid salt, if present) and acetic acid be liberated.

Into a flask of 150 or 200 cc. capacity introduce from a pipette 50 cc. of the solution of sodium phosphate, add 5 cc. of the solution of sodium acetate, heat to the boiling point and titrate the hot solution with the uranium solution from a burette until a precipitate ceases to form. Transfer a drop of the hot mixture by means of a stirring rod to a porcelain dish, in which there is a drop of the solution of potassium ferrocyanide, and when the two drops are brought together, if no dark coloration takes place, heat the mixture again, titrate with the uranium solution and test as before. Repeat the process until by testing a dark color is produced. When an agreement is reached, the uranium solution is diluted so that 20 cc. corresponds to 50 cc. of the sodium phosphate solution. If, for example, 18.2 cc. of the uranium solution is sufficient, the addition of 1.8 cc. water would be required, and 50 cc. would require 49.4 cc. water (18.2 : 1.8 : : 500 : x). 1 cc. of the standardized uranium solution corresponds to 0.005 grm. P_2O_5.

DETERMINATION OF THE PHOSPHORIC ACID OF THE PHOSPHATES.

If the urine is not clear it is filtered, or, if the urine is alkaline, neutral, or slightly acid, and it is desired to include the phosphoric acid of the sediment in the estimation, render the urine

strongly acid with acetic acid; mix well by stirring with a glass rod and filter, if necessary. Introduce 500 cc. of the urine in a beaker, add 10 cc. solution ammon. chloride (1 part ammon. chloride and 10 parts water) and 40 to 50 cc. magnesium mixture, when 100 cc. strong ammon. hydrate is added and the fluid well mixed with a stirring rod. Having stood 6 to 12 hours, filter, and transfer the precipitate to the filter by means of a stirring rod provided with a small piece of rubber tubing placed on its end. Wash the precipitate with water containing one-third its volume of ammon. hydrate. The washing is continued until some of the wash water, having been boiled in a test tube to drive off the excess of ammonia, and rendered acid with nitric acid, ceases to yield a turbidity with a solution of silver nitrate. When the precipitate is washed, it is immediately transferred to a graduated 250 cc. flask. This is brought about by perforating the filter with a glass rod, and with a fine stream of water from a wash bottle every trace of the precipitate is removed from the paper. Dissolve the precipitate with acetic acid, and with water fill to the mark. Mix well by shaking. With a pipette introduce 50 cc. of the solution into a 200 cc. flask, add 5 cc. of the solution of sodium acetate, heat to the boiling point (preferably on a sand bath), and from a burette containing the uranium solution titrate while hot and test with the solution of potassium ferrocyanide as in standardizing the uranium solution. Titrations of other portions of 50 cc. are made until an agreement is reached.

DETERMINATION OF THE TOTAL QUANTITY OF PHOSPHORIC ACID.

The quantity of P_2O_5 in glycerin-phosphoric acid, as well as that of the phosphates, is determined by boiling the urine, having been acidified with nitric acid. For this purpose 250 cc. urine is rendered strongly acid with nitric acid and boiled thirty minutes, and, when cold, the phosphoric acid is precipitated by rendering the solution alkaline with ammon. hydrate and treating with 50 cc. magnesium mixture and ammon. hydrate, when the estimation is continued as above in determining the quantity of P_2O_5 of the phosphate.

CALCULATION.

20 cc. of the uranium solution corresponds to 0.1 grm. P_2O_5. If more or less than 20 cc. of the uranium solution were employed for 50 cc. of the solution, 25 cc., for example, the equation would be 20 : 0.1 : : 25 : x. x = the weight of P_2O_5 in 50 cc. of the solution, or multiply the number of cc. uranium solution employed in the titration by 0.005, the product of which is the weight of P_2O_5 in 50 cc. of the solution. The number 0.005 is the weight in grms. of P_2O_5, which corresponds to 1 cc. of the uranium solution. As the volume of the solution titrated corresponds to the volume of urine, multiply the number of grms. P_2O_5 found in 50 cc. by two, the product of which is the per cent. of P_2O_5 in the urine, not taking into consideration the specific gravity of the urine. The difference between the weight of P_2O_5 of the phosphates and the weight of the total quantity of P_2O_5 in the urine is the weight of P_2O_5 of the glycerin-phosphoric acid.

SULPHUR COMPOUNDS.

For the combinations of sulphur in the urine, refer to page 36.

The estimations of sulphur and sulphuric acid in the urine are (1) the quantity of sulphuric acid of both sulphates and ester compounds; (2) the quantity of sulphuric acid of ester compounds, and (3) the quantity of sulphur of compounds other than sulphates and esters.

SULPHURIC ACID OR SULPHUR OF SULPHATES AND ESTER COMPOUNDS.

THE GRAVIMETRIC METHOD.

The gravimetric method of determining the quantity of sulphuric acid or sulphur in the urine differs in some respects from that employed in estimating it in aqueous solutions, as the barium sulphate forming in the urine is liable to incorporate bodies which cannot be separated by washing with water. It is, therefore, recommended to dilute 50 cc. urine to 150 cc. with water before precipitating with barium chloride. If the urine is not clear, it is filtered through a dry filter into a dry flask before diluting, when 50 cc. is introduced into a 250 or 300 beaker with a pipette and diluted with 100 cc. water. Acidify with a

small quantity of hydrochloric acid, heat on a wire gauze until the fluid begins to boil, and precipitate with a clear solution of barium chloride (1 part barium chloride and 10 parts water). When precipitated, place the beaker on a water bath; the precipitate having settled, determine if the precipitation is complete by introducing a small quantity of the solution of barium chloride into the warm fluid with a pipette; if no cloudiness is produced, enough of the barium solution has been added. If, on the other hand, a precipitate forms, heat again on a wire gauze and add more of the solution of barium chloride. Complete precipitation having taken place, the mixture is heated one or two hours on a water bath, and filtered by decanting through a small, fine filter paper (preferably washed Swedish filter paper or No. 590 Schleicher and Schüll's filter paper). When the fluid has been decanted, except 20 or 30 cc., add about 50 cc. distilled water, stir with a glass rod without touching the glass, heat again on the water bath until the precipitate has settled, and filter as before. Collect the precipitate on the filter, and remove every trace that may remain in the beaker with a stirring rod provided with a small piece of rubber tubing placed over its end. Wash with distilled water until the wash water ceases to yield a precipitate with a solution of silver nitrate, then continue the washing with about 100 cc. alcohol heated in a flask to the boiling point. The alcohol will remove some of the organic matter insoluble in water. With the precipitate there are still impurities, to separate which (Brüggelmann) dry the filter with precipitate at 100° C., and transfer the precipitate to a platinum crucible or small platinum dish and ash the paper on a platinum wire. The precipitate, with the ash, is moistened with concentrated hydrochloric acid, when 3 or 4 cc. water is added, and the mixture well stirred with a short stirring rod. Warm gently over the free flame and decant the fluid through a small filter. Repeat this process five times, then transfer the precipitate to the filter and wash with water until the wash water ceases to yield a precipitate or cloudiness with a solution of silver nitrate. Dry the precipitate with filter at 100° C. and ash the paper, and into a weighed platinum crucible introduce the precipitate and ash, and heat ten minutes with a Bunsen's burner or Argand spirit lamp. After cooling, moisten with dilute sulphuric acid and heat gradually to dull

redness, place in a desiccator and, when cool, weigh. The treatment with dilute acid is, to transform any barium sulphide, that may have formed, into the sulphate.

CALCULATION.

The molecular weight of $BaSO_4$ is 233, that of H_2SO_4 is 98; the atomic weight of sulphur is 32, therefore,

$$233 : 98 :: \overset{\text{Wt. of } BaSO_4}{} : x.\ x = \text{weight of } H_2SO_4 \text{ and}$$

$$233 : 32 :: \overset{\text{Wt. of } BaSO_4}{} : x.\ x = \text{weight of Sulphur.}$$

The quantity of H_2SO_4 or sulphur found, is from 50 cc. urine, therefore, for 100 cc. multiply by two.

THE VOLUMETRIC METHOD.

WILDENSTEIN, BRÜGGELMANN AND NEUBAUER.

The solutions required for the volumetric method of estimating sulphuric acid are one-fifth normal solutions of barium chloride and potassium sulphate, and the apparatus required is a special filter besides burettes.

ONE-FIFTH NORMAL BARIUM CHLORIDE.

Pure barium chloride, containing water of crystallization, is dried by pressing between filter paper, weighed on a balance of precision, put into a clean dry flask of suitable size, and water is added, so that in 1000 cc. of the solution there are 24.392 grms. barium chloride. The quantity of water in which to dissolve the salt is determined by the equation—

$$24.392 : 1000 :: \overset{\text{No. grms. Ba salt weighed}}{} : x.\ x = \text{No. cc. water required.}$$

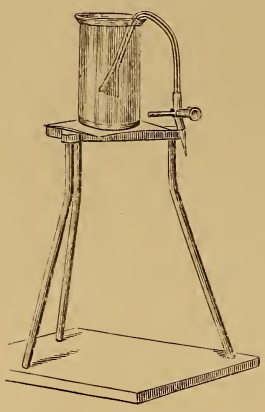
ONE-FIFTH NORMAL POTASSIUM SULPHATE.

To prepare the solution of potassium sulphate, the salt, chemically pure, is dried at 100° C., and, having cooled in a desiccator, it is weighed and introduced into a clean, dry flask. Add water to the salt in quantity until the strength of the solution is 17.4 grms. potassium sulphate in 1000 cc. of the solution. From the weight of the salt the quantity of water in which to dissolve the salt is determined by the equation—

$$17.4 : 1000 :: \overset{\text{No. of grm. } K_2SO_4 \text{ weighed}}{} : x.\ x = \text{No. cc. water required.}$$

The special or syphon filter required is represented by Fig. 34. It is made of a piece of combustion tubing, the internal diameter of which is 12 to 15 mm., and drawn out from 15 to 20 mm. of one end by heating in a blast lamp successive parts until the length of the drawn-out part is 17 cm. long, then the tube is bent about 8.5 cm. from the bell-shaped end, as seen by the Fig. A small rubber tube passes over the lower end of the syphon and extends to a lower plane than the end with the filter. The rubber tube is secured by a pinch-cock. The filter in the bell-shaped end of the tube is made of well-washed filter paper. One layer is placed over the constricted part, followed by fragments of wet paper. Before using, water is drawn through the filter to ascertain if the packing is loose enough to permit the passage of water, and if it is found that the water passes through with difficulty, less material is employed or the fragments of paper are loosened.

Fig. 34.



THE TITRATION.

100 cc. of the filtered urine is introduced into a beaker of 200 to 300 cc. capacity and rendered acid with 2 cc. hydrochloric acid, sp. gr. 1.12. The beaker is then placed on a wire gauze or sand bath and heated to the boiling point, when portions of 1 cc. of the one-fifth normal barium chloride are added from a 25 cc. burette until, after mixing with a stirring rod, no further precipitation appears to form. To the end of the process the temperature of the mixture is kept at the boiling point. The end of the syphon with the filter is introduced into the mixture, and while the other extremity is raised and turned, a glass tube is introduced into the rubber tube, and the urine is drawn by suction through the filter nearly to the end of the syphon, when the pinch-cock is attached and the filter is ready for use. Filter about 2 cc. of the fluid into a test tube and add a few drops of the barium solution from the burette. If a precipitate forms, the fluid

in the test tube is returned to the beaker, and the test tube is rinsed with water two to three times and the rinsings added to the fluid in the beaker. Add 1 cc. of the barium solution, mix well with a stirring rod, filter 3 or 4 cc., but instead of testing this filtrate, return it to the beaker, as this quantity of fluid may have been in the tube during the last titration. Having mixed well, filter another portion and test with the barium solution; if a precipitate or cloudiness is produced, repeat with 1 cc. of the barium solution until a precipitate is no longer produced. Having determined about how much of the barium solution is required for 50 or 100 cc. urine, empty and clean the beaker, wash the filter with distilled water and fill the burette to the 0 mark with the barium solution. Introduce the same quantity of urine and acid into the beaker, heat and titrate with 1 cc. less of the barium solution than was employed before. Stir, filter, and test the filtrate with 0.1 cc. of the barium solution. If no cloudiness is produced, titrate with 0.1 cc. of the barium solution, and continue the titrations, adding 0.1 cc. of the barium solution both for titrating and testing, until the filtrate, when tested, becomes cloudy after the lapse of a few seconds, when another portion is filtered and tested with the one-fifth normal potassium sulphate, and if a precipitate is produced immediately, too much of the barium chloride solution has been added, in which case another titration is made with the same quantity of urine, but employing 0.3 cc. less of the solution of barium chloride, and if, by testing, a light precipitate forms at once, titrate with 0.1 cc. of the barium solution until a light precipitate forms in the filtrate several seconds after the addition of the solution of barium chloride. When this takes place, another portion is filtered and tested with the solution of potassium sulphate, and if the fluid becomes somewhat turbid in 15 to 20 seconds, the exact quantity of the solution of barium chloride required by the sulphuric acid in the urine has been employed.

CALCULATION.

Multiply the number of cc. of the one-fifth normal barium chloride employed by 0.0098; the product is the number of grms. H_2SO_4; or, for the quantity of sulphur in the quantity of urine titrated, multiply the number of cc. of the one-fifth normal barium chloride employed by 0.0032.

SULPHURIC ACID OF THE ESTER COMPOUNDS.

For the estimation of sulphuric acid of the ester compounds in the urine, that of the sulphates is first separated. For this purpose saturated solutions of barium hydrate and barium chloride are prepared, when two volumes of the former are mixed with one volume of the latter. 100 cc. of the urine and 100 cc. of the barium solution or mixture are introduced into a dry beaker and well mixed with a stirring rod, and in 10 or 15 minutes the solution is filtered through a dry filter into a dry beaker. Introduce 100 cc. of the filtrate into a beaker, render strongly acid with hydrochloric acid and heat to the boiling point, then proceed in estimating the sulphuric acid by the gravimetric method, as above.

SULPHUR NOT IN SULPHATES OR ESTER COMPOUNDS.

To determine the quantity of sulphur in the urine, not in sulphates or ester compounds, estimations are made of the sulphur of the latter compounds, as above, by the gravimetric or volumetric method, and of the total quantity of sulphur in the urine, and from the weight of the latter the weight of the former is subtracted; the remainder is the weight of the sulphur in compounds, the constitution of the greater number of which is unknown.

To estimate the total quantity of sulphur in urine, introduce 50 cc. of the urine into a platinum dish, render strongly alkaline with a solution of sodium carbonate and add 3.5 grms. potassium nitrate, when the dish is placed on a water bath and the evaporation continued until the water is driven off. Heat is then applied gradually until the residue fuses, avoiding any higher temperature than necessary to bring about fusion. If the fusion is still dark in color, small crystals of potassium nitrate are added and the heat continued until it becomes white. When cold, treat the residue with water and separate the insoluble part $BaCO_3$ by filtering, wash with water and add wash water to the filtrate. The residue is washed until the wash water ceases to change the color of turmeric paper. To transform the nitrates and nitrites in the filtrate and wash water, evaporate in a porcelain dish on a water bath with an excess of hydrochloric acid. To drive off the nitric

and nitrous acids, the process is repeated, with the addition of a small quantity of hydrochloric acid. Dissolve the residue in water, transfer the solution to a beaker, rinse the dish several times with water and add the rinsings to the fluid in the beaker. The sulphuric acid in the fluid may be estimated either by the volumetric or gravimetric method; if by the latter, the process may be shortened somewhat, as it is not necessary to wash the $BaSO_4$ with alcohol or treat it with dilute sulphuric acid.

CALCULATION.

For the calculation of the quantity of sulphur from the weight of $BaSO_4$, refer to calculation of the gravimetric method. If the volumetric method has been employed, for the quantity of sulphur, multiply the weight of H_2SO_4 by $\frac{32}{98}$. The weight of the sulphur of the sulphates and ester compounds in 50 cc. of the urine having been determined, it is subtracted from the weight of the total quantity of sulphur in 50 cc. of the urine; the remainder is the weight of sulphur of compounds other than sulphates and esters in 50 cc. of the urine.

CHLORINE.

VOLHARD'S METHOD, MODIFIED BY SALKOWSKI.

The chloride and sulphocyanide of silver are insoluble in water and dilute nitric acid, while ferric sulphocyanide is soluble and imparts a red color to its solutions. Reactions by which these bodies are formed are understood by the equations:—

$$NaCl + AgNO_3 = NaNO_3 + AgCl.$$
$$AgNO_3 + NH_4(CNS) = NH_4NO_3 + Ag(CNS).$$
$$Fe_2(SO_4)_3 + 6NH_4(CNS) = 3(NH_4)_2SO_4 + Fe_2(CNS)_6.$$

A definite quantity of urine, having been rendered strongly acid with nitric acid, is treated with an excess of a solution of silver nitrate, the strength of which is known when the silver chloride is separated by filtering, and the quantity of silver in the filtrate determined by titrating with a standardized solution of ammon. sulphocyanide. From the quantity of silver in the solution added, the quantity found in solution is subtracted; the remainder is the quantity of silver with which the chlorine in the urine has combined. If the titration with ammon. sulphocyanide be carried on without the separation of silver chloride,

the results are unreliable, as the silver chloride reacts on silver sulphocyanide, causing the quantity of ammon. sulphocyanide to vary. In titrating with ammon. sulphocyanide, the ferric salt is the indicator.

PREPARATION OF SOLUTIONS.

SOLUTION OF SILVER NITRATE.

Dissolve pure crystallized silver nitrate in distilled water, so that the strength of the solution is 29.075 grms. in 1000 cc. The quantity of water in which to dissolve a weighed quantity of silver nitrate is ascertained by the equation of 29.075 : 1000 : : No. of grms. $AgNO_3$ weighed : x or number of cc. water. The silver nitrate, when weighed, is put into a dry flask or bottle provided with a glass stopper, and the required amount of water is added. 1 cc. of the silver solution contains the quantity of silver which will combine with the chlorine of 0.01 grm. sodium chloride or 0.00606 grm. chlorine.

SOLUTION OF FERRIC ALUM.

Ferric sulphate may be employed as well as ferric alum, but as the ammon. sulphate in the latter takes no part in the reaction, and as the compound is generally obtained free of chlorine, it is preferred. A saturated solution is employed, which is prepared by dissolving 50 grms. in 1000 cc. distilled water.

SOLUTION OF AMMONIUM SULPHOCYANIDE.

Dissolve about 7 grms. ammon. sulphocyanide in 1100 cc. distilled water, and having mixed well by shaking, fill a 25 cc. burette with the solution to the 0 mark. For the purpose of standardizing this solution with the solution of silver nitrate, 10 cc. of the silver solution is introduced into a beaker and diluted with about 100 cc. water, and to the fluid 4 cc. nitric acid, sp. gr. 1.2, and 5 cc. of the solution of ferric alum are added; and having mixed well with a stirring rod, the fluid is titrated with the solution of ammon. sulphocyanide, until, by the addition of 0.1 cc., a red color or tint is imparted to the fluid, recognized by placing the beaker on a white background. Titrations are repeated until an agreement is reached, when 1000 cc. of the solution is diluted so that 25 cc. corresponds to 10 cc. of the

silver solution. For example: 21.3 cc. of the sulphocyanide solution was required to produce the red color with 10 cc. of the silver solution; the addition of 3.7 cc. water to 21.3 cc. of the solution would be required to dilute the solution to 25 cc., and to dilute 1000 cc. of the solution 173.7 cc. water would be required (21.3 : 3.7 : : 1000 : x or 173.7).

TITRATION OF THE URINE.

Into a 100 cc. graduated flask, provided with a glass stopper, introduce 10 cc. urine, 50 cc. water, 4 cc. nitric acid, sp. gr. 1.2, and 15 cc. of the solution of silver nitrate. Having closed the flask with the stopper, shake several minutes, and with a fine stream of water from a wash bottle rinse the end of the stopper and fill the flask with water to the mark. Close the flask again and mix well by shaking, and after settling, filter through a dry filter paper into a dry graduated 80 cc. flask. In filtering, the clear fluid is carefully decanted into the filter without agitating the precipitate. When the filtrate reaches the mark of 80 cc., the filter is removed, the filtrate introduced into a 300 cc. flask, and the flask rinsed with water several times and rinsings added to the filtrate. Add 5 cc. of the solution of ferric alum to the fluid and titrate with the solution of ammon. sulphocyanide from a burette, until, after having mixed well by agitating the flask, a permanent red color or tint is imparted to the fluid by the addition of 0.1 cc. of the solution.

CALCULATION.

In the fluid titrated there was introduced 15 cc. of the solution of silver nitrate. If this quantity of silver solution were titrated with the solution of ammon. sulphocyanide in the absence of chlorides, 37.5 cc. of the sulphocyanide solution would be required, but chlorine of the urine having combined with some of the silver, less of the sulphocyanide solution would be required. The quantity of chlorine which has combined with silver is determined by first estimating the quantity of silver in excess or that remaining in solution by titrating the filtrate with the solution of ammon. sulphocyanide and subtracting the number of cc. required from 37.5, the remainder is equivalent to a definite quantity of

silver or chlorine. Example: 5.9 cc. of the solution of ammon. sulphocyanide was required for the silver in the filtrate which represents eight-tenths of the urine taken, therefore, for the filtrate and wash water of the 10 cc. urine, 7.37 cc. of the sulphocyanide solution would be required ($\frac{10 \times 5.9}{8} = 7.37$). As 15 cc. of the silver solution employed requires 37.5 cc. (10 : 25 : : 15 : 37.5) of the ammon. sulphocyanide solution, and after coming in contact with the urine only 7.37 cc. was required; therefore, chlorine equivalent to 30.1 cc. of the sulphocyanide solution combined with the silver, which is in 12 cc. of the solution of silver nitrate (37.5 : 15 : : 30.13 : 12). That is, in 10 cc. urine there is sufficient chlorine to combine with the silver in 12 cc. of the standardized solution of silver nitrate, and as 1 cc. of the solution corresponds to 0.00606 grm. chlorine, 12 cc. would correspond to 0.07272 grm. There being this quantity of chlorine in 10 cc. of the urine, in 100 cc. there would be 0.7272 grm. chlorine.

NEUBAUER'S METHOD.

By Neubauer's method the quantity of the chlorine in urine is estimated by titrating with a solution of silver nitrate of known strength; the indicator employed is a solution of the yellow chromate of potassium. The chlorine having combined with silver, and with a slight excess of silver solution silver chromate is formed, which imparts a red color or tint to the mixture, but before titrating the organic matter of the urine is oxidized. For this purpose introduce 10 cc. of the urine into a platinum dish or crucible, to which add 1 grm. sodium carbonate and about 3 grms. potassium nitrate—both free of chlorides—and evaporate to dryness on a water bath. When dry, place the dish or crucible on a triangle support, and with a small flame of a gas or spirit lamp heat gradually by keeping the flame in lateral movement, so that the heat be applied equally. Increase the heat until the residue melts, forming a white mass when the lamp is removed. Dissolve the mass, when cold, in water, and empty into a 150 cc. flask. Before transferring the fluid, place a small funnel in the neck of the flask, so as to prevent loss. Rinse the dish and funnel and add the rinsings to the fluid, neutralize the solution by first rendering distinctly acid with dilute nitric acid and neutralizing with a solution of sodium carbonate. To the

neutral solution add two or three drops of a saturated solution of the yellow chromate of potassium, when it is prepared for titrating. The solution of silver nitrate of the strength employed in Volhard's method of estimating chlorine in the urine is preferred. For the preparation of this solution, refer above. The neutralized solution is titrated with the solution of silver nitrate from a burette until, after mixing well by agitating the flask, the mixture is changed to a red color or tint by the addition of 0.1 cc.

CALCULATION.

For the weight of chlorine or sodium chloride in grms., multiply the number of cc. of the silver solution employed in the titration by 0.00606 for chlorine, and by 0.01 for NaCl; the product of each multiplied by 10 will give the number of grms. in 100 cc. urine.

REMARKS.

In Volhard's method of estimating chlorine in the urine, potassium sulphocyanide may be employed instead of the ammonium salt. The solution of either salt undergoes no change by keeping in well filled glass-stoppered bottles, in a cool, dark place. If the nitric acid, sp. gr. 1.2, is colored, heat in a flask on a water bath until the nitrous fumes are driven off.

The most difficult part of Neubauer's method is to neutralize the solution of the fused mass. If, however, too much of the solution of sodium carbonate be added after having rendered acid with nitric acid, the solution may be rendered slightly acid with acetic acid, or the chlorine may be estimated by Volhard's method. With this in view, render the solution strongly acid with nitric acid, add 15 cc. of the silver solution, boil 15 to 20 minutes, to drive off the nitrous acid, and proceed as with urine treated with acid and silver solution.

POTASSIUM AND SODIUM.

In the estimation of potassium and sodium in the urine, chlorides of these metals are weighed; but before this, the phosphoric and sulphuric acids are separated, the organic matter oxidized, and the calcium magnesium and also barium employed in separating the acids are removed.

50 cc. of the urine is mixed with 50 cc. barium mixture* in a dry beaker. Having stood three or four hours, filter into a dry, graduated 100 cc. glass cylinder, and when the filtrate reaches the mark of 80 cc., remove the filter. Evaporate the filtrate 80 cc. with the rinsings of the cylinder in a platinum dish to dryness on a water bath, and when evaporated, transfer the dish to a triangle support, and heat gently with a small flame. If not heated gradually, by the sudden evolution of steam or by the rapid oxidation of organic matter, loss may result. A high temperature is avoided, as the chlorides volatilize at a white heat. When cold, treat the residue in the dish with 20 cc. water and render the solution acid with dilute hydrochloric acid, then the fluid is rendered alkaline with ammonium hydrate and a solution of ammonium carbonate added until a precipitate ceases to form. The metals of the alkaline earths separate as carbonates. Filter, and wash the precipitate with water and add the wash water to the filtrate. Evaporate the filtrate and wash water in the platinum dish to dryness on a water bath, and heat the residue with great care, to avoid crepitation of the chlorides. Repeat the process of dissolving in a small quantity of water, rendering alkaline with ammon. hydrate, adding a small quantity of a solution of ammon. carbonate, filtering, washing, evaporating and heating the residue until ammon. carbonate ceases to produce a precipitate, when the residue is heated in a weighed platinum dish provided with a cover, and having cooled in a desiccator, it is weighed. The difference in the weight of the empty dish and the weight of the dish + residue is the weight of potassium and sodium chlorides in 40 cc. of the urine. The chlorides in the dish are dissolved in a small quantity of water, and the solution transferred to a porcelain dish and the dish rinsed with water, the rinsings added to the solution in the porcelain dish. To the solution add an excess of a solution of platinum chloride (1 part $PtCl_4$ and 10 parts water) and evaporate on a water bath, but the evaporation should not be carried so far that water of crystallization of compounds is driven off. Treat the residue with a few drops of the platinum water to dissolve the sodium compound, and add a

* Composed of 2 vols. cold saturated solution of pure barium hydrate and 1 vol. cold saturated solution of barium chloride.

mixture of 4 vols. absolute alcohol and 1 vol. ether; with a glass rod stir several minutes, let stand in a cool place 30 minutes, and filter through a small, dry filter paper and wash well with the mixture of alcohol and ether. When washed, dissolve the yellow precipitate on the paper with hot water, and, solution having taken place, wash with hot water until the platinum compound is washed from the paper. Evaporate the filtrate and wash water in a weighed platinum dish provided with a cover on a water bath, and dry the residue at 100° C. in an air or steam bath, and after cooling in a desiccator, it is weighed.

CALCULATION.

Multiply the weight of the platinic potassium chloride by 0.3055; the product is the weight of potassium chloride in the urine. Having determined the weight of potassium and sodium chlorides, the weight of potassium chloride is subtracted from it, the remainder is the weight of sodium chloride. To determine the weight of potassium, multiply the weight of potassium chloride by 0.5246. The weight of sodium is determined by multiplying the weight of sodium chloride by 0.3938. As the quantities are of 40 cc. urine, multiply by 2.5; the product is the weight of each in 100 cc. of the urine.

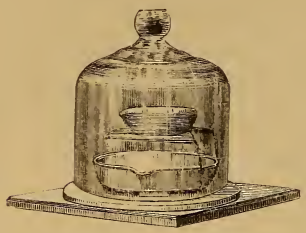
AMMONIA.

SCHLÖSING AND NEUBAUER'S METHOD.

When a salt of ammonium in solution comes in contact with calcium hydrate, ammonia gas is set free ($(NH_4)_2CO_3 + Ca(OH)_2 = CaCO_3 + 2H_2O + 2NH_3$). When ammonia comes in contact with dilute sulphuric acid, ammon. sulphate is formed ($2NH_3 + H_2SO_4 = (NH_4)_2SO_4$). The ammonia, in a certain volume of urine, is liberated by the action of calcium hydrate, and is absorbed by dilute sulphuric acid of known strength, and by titrating with one-fifth normal potassium hydrate the quantity of ammonia combined with the acid is determined. For the preparation of normal sulphuric acid, refer to page 112, and for the preparation of normal potassium hydrate, refer to page 109. From the normal solutions the one-fifth of each is prepared by introducing 100 cc. into a 500 cc. graduated flask and filling with water to the mark. Before using, the solutions are mixed by shaking. A

saturated solution of calcium hydrate and a solution of litmus are also required. Calcium hydrate is mixed with water, and after standing one or two hours and frequently shaking, the solution is filtered and kept in a well-stoppered bottle. For the preparation of a solution of litmus paper, refer to page 114. Besides these solutions, the apparatus represented by Fig. 35 is required. The apparatus comprises a bell jar or receiver with ground margin, a glass plate with ground surface, on which the receiver is placed to prevent the escape of ammonia, two evaporating dishes and a tripod. The evaporating dishes should be shallow. The internal (transverse) diameter of the bell jar is 14 cm.

Fig. 35.



30 cc. of the one-fifth normal sulphuric acid is introduced into one of the evaporating dishes, and a sufficient quantity of the solution of litmus is added to impart a distinct red color to the fluid. Into the other evaporating dish introduce 20 cc. of clear or filtered urine and 20 cc. of the saturated solution of calcium hydrate. The dish is then placed on the glass plate under the tripod supporting the other dish containing the dilute acid, when, with some grease, the receiver is fixed in place. After standing three days in a cool place where the temperature is not subject to variation, empty the dish containing the dilute acid into a beaker, and with water rinse the dish several times, adding the rinsings to the fluid in the beaker. With the one-fifth normal potassium hydrate in a burette, titrate the acid solution until, after stirring with a glass rod, the fluid turns purple or blue by the addition of 0.1 cc.

CALCULATION.

In the absence of ammonia, 30 cc. of the one-fifth normal acid would require 30 cc. of the one-fifth normal potassium hydrate, but by the absorption of ammonia some of the acid is neutralized, and, therefore, less of the potassium hydrate is required. If, for example, 27.5 cc. of the one-fifth normal potassium hydrate be required to neutralize the 30 cc. one-fifth normal sulphuric acid employed, 2.5 cc. of the acid is in combination with ammonia.

1 cc. of the one-fifth normal sulphuric acid will combine with 0.0034 grm. ammonia and 2.5 cc. of the acid corresponds to 0.0085 grm. ammonia ($2.5 \times 0.0034 = 0.0085$). There being 0.0085 grm. ammonia in 30 cc. urine, in 100 cc. there is 0.02833 grm. ammonia. ($\frac{0.0085 \times 100}{30} = 0.02833$.)

CALCIUM AND MAGNESIUM OXIDES.

These bodies in the urine are estimated by the ordinary gravimetric methods employed in estimating them in inorganic compounds; besides, they are estimated by volumetric processes. Calcium is precipitated as an oxalate with a solution of ammon. oxalate in the presence of magnesium and phosphoric acid, if the solution be rendered alkaline with ammon. hydrate and then acidified with acetic acid. Calcium is separated from the urine in the form of an oxalate in the volumetric as well as in the gravimetric method. The magnesium is precipitated from an acid solution in the presence of a soluble phosphate by rendering the solution alkaline with ammon. hydrate as magnesium ammon. phosphate ($MgNH_4PO_4$). After the separation of calcium from the urine by means of ammon. oxalate, the urine contains all of the reagents necessary, when rendered alkaline with ammon. hydrate, to form the insoluble magnesium ammon. phosphate, but the precipitation is facilitated by the presence of ammon. hydrogen phosphate. The magnesium is precipitated in this form in both the gravimetric and volumetric methods.

CALCIUM OXIDE.

THE GRAVIMETRIC METHOD.

250 cc. clear or filtered urine is introduced into a beaker and treated with ammon. hydrate until a precipitate is formed, when it is rendered acid with acetic acid. The phosphates of calcium and magnesium, which precipitate by rendering the urine alkaline, are dissolved by the acetic acid. Place the beaker on a wire gauze and heat until the fluid begins to boil, when the calcium is precipitated by adding an excess of a saturated solution of ammon. oxalate. Mix well by stirring with a glass rod, avoiding contact with the surfaces of the beaker. Place the beaker on a water bath and let remain until the precipitate has

settled. Introduce 1 or 2 cc. of the ammon. oxalate solution into the clear fluid from a pipette; if no precipitate is produced a sufficient quantity has been added. Filter through a filter paper free of ash, and collect the precipitate by means of a glass rod, over the end of which a short piece of rubber tubing is placed. Wash the precipitate with distilled water until the wash water remains clear when tested with a solution of silver nitrate and a few drops of nitric acid. Preserve the filtrate and wash water for the estimation of magnesium oxide. Dry the filter with precipitate at 100° C., and when dry, place into a weighed platinum crucible and heat with a gas or spirit lamp in the open crucible. The filter having been ashed, place the lid on the crucible and heat with the blast lamp at a high temperature 10 or 15 minutes, and the crucible having cooled in a desiccator, it is weighed. The process of heating and weighing should be repeated until the weight becomes constant. The difference between the weight of the empty crucible and that of the crucible and contents is the weight of calcium oxide in 250 cc. of the urine. By employing the equation $250 : \overset{\text{Wt. of}}{CaO} :: 100 : x$, the weight of calcium oxide in 100 cc. is determined.

THE VOLUMETRIC METHOD—NEUBAUER.

By this method the calcium is separated from the urine, as in the gravimetric method, and by heat it is transformed into the oxide, when it is treated with an excess of dilute hydrochloric acid of known strength, after which the quantity of free hydrochloric acid is estimated by titrating with a solution of potassium hydrate, and by calculation the quantity of calcium oxide is determined. One-half normal hydrochloric acid and potassium hydrate are employed. For the preparation of normal hydrochloric acid, refer to page 113, and for the preparation of normal potassium hydrate, refer to page 109. A one-half normal solution is prepared by mixing one volume of the normal solution with one volume distilled water. This is accomplished without difficulty by introducing 250 cc. of the normal acid or potassium hydrate into a 500 cc. graduated flask and filling with water to the mark. The calcium in 250 cc. urine having been precipitated in the form of oxalate, as in the gravimetric method given above, and the precipitate having been washed, dried, the filter ashed

and the oxalate decomposed by heat into the oxide, the latter is transferred from the crucible to a 200 cc. flask by means of a hair pencil, when to the oxide 50 cc. water is added, and after mixing well by agitating the flask, 10 cc. of the one-half normal hydrochloric acid is introduced into the mixture from a burette. Calcium oxide will dissolve in the acid, and by heating the solution any carbonic acid present is driven off. Add a sufficient quantity of the solution of litmus to impart a distinct red color to the fluid. From a burette containing the one-half normal potassium hydrate, titrate the fluid until, after mixing well by agitating the flask, the color of the solution changes purple or blue by the addition of 0.1 cc.

CALCULATION.

10 cc. of the one-half normal potassium hydrate would be required to neutralize the 10 cc. one-half normal hydrochloric acid if no base were present, but as calcium oxide combines with the acid, less of the potassium hydrate would be required. If, for example, 7.5 cc. of the potassium hydrate solution neutralized the acid, 2.5 cc. of the acid was absorbed by the calcium oxide, and as 1 cc. of the half normal hydrochloric acid will combine with 0.014 grm. calcium oxide, therefore, there is in 250 cc. of the urine 0.035 grm. calcium oxide ($0.014 \times 2.5 = 0.035$), and in 100 cc. 0.014 grm.

MAGNESIUM OXIDE.

GRAVIMETRIC METHOD.

For the estimation of the quantity of magnesium oxide, the filtrate and wash water of the calcium oxalate from 250 cc. urine are employed. Refer above to gravimetric method, page 161. Evaporate the fluid in an evaporating dish to about 200 cc., transfer to a beaker, rinse the dish with water and add rinsings to the fluid in the beaker. To the fluid add one-fourth its volume strong ammonium hydrate and 5 or 10 cc. of a clear solution of sodium ammon. hydrogen phosphate (1 part of the salt and 10 parts water), the magnesium is precipitated as magnesium ammon. phosphate. Having mixed well with a stirring rod without the rod coming in contact with the beaker, let stand in a warm place, protected from dust, 12 to 24 hours, when the solu-

tion is filtered through a filter paper free of ash. Collect the precipitate by means of a glass rod provided with a short piece of rubber tubing placed over its end, and wash the precipitate on the filter with a mixture of 2 vols. water and 1 vol. ammon. hydrate until the wash water yields no precipitate, after having been boiled to drive off ammonia acidified with nitric acid and treated with a solution of silver nitrate. Dry the filter with precipitate at 100° C. and transfer from the paper to a clean, dry watch glass as much of the precipitate as is possible, and place the filter in a weighed platinum crucible, and ash it by heating the crucible with a gas or spirit lamp to redness. When the ash has become white or gray, remove the lamp and transfer the precipitate from the watch glass to the crucible by means of a fine hair pencil, and with the crucible closed with the lid heat gradually to redness. If the precipitate, after having been heated, is not white, it is due to the presence of carbon of organic matter, to oxidize which, introduce a crystal of pure ammon. nitrate into the crucible and heat again, or oxidation will take place by heating in the open crucible to redness 30 minutes. Having cooled in a desiccator, weigh. The body weighed is magnesium pyrophosphate; the magnesium ammon. phosphate having been decomposed by heat forming the pyrophosphate, as shown by the equation, $2MgNH_4PO_4 = Mg_2P_2O_7 + H_2O + 2NH_3$.

CALCULATION.

Multiply the weight of the precipitate by 0.36036; the product of which is the weight of magnesium oxide in 250 cc. of the urine, and two-fifths of which is the quantity in 100 cc. urine.

THE VOLUMETRIC METHOD—NEUBAUER.

The calcium having been separated from 250 cc. urine, as by the gravimetric method of estimating calcium, page 161, and the magnesium precipitated as magnesium ammon. phosphate from the filtrate and wash water as by the gravimetric method of estimating magnesium, page 163, and the precipitate having been well washed, it is transferred to a beaker by perforating the filter with a glass rod and by means of a fine stream of water from a wash bottle. When the precipitate is removed from the paper, it is dissolved with dilute acetic acid, and the phosphoric

acid in the solution is determined by titrating with a standardized solution of uranium acetate. For the preparation of the uranium solution and the method of titrating, refer to pages 144 and 145.

CALCULATION.

Multiply the weight of phosphoric acid, P_2O_5, found by 0.5633 ; the product of which is the weight of magnesium oxide in 250 cc. of the urine, and two-fifths of which is the quantity in 100 cc. urine.

CHAPTER XII.

Albumen, Scherer's Method—Globuline, Hammarsten's Method—Pohl's Method—Hemialbumose, Gravimetric and Optical Methods—Peptone, Optical Method—Remarks on the Estimation of Albuminous Bodies—Sugar, Fehling's Method—Fehling's Method, Modified by Pavy—Roberts' Method—Optical Method—Remarks on the Estimation of Sugar in the Urine.

ALBUMEN.

SCHERER'S METHOD.

Urine in which albumen is to be estimated, if not clear, is filtered. Into a beaker of about 200 cc. capacity, 100 cc. urine is introduced. If the reaction of the urine is not strongly acid, add acetic acid until the reaction is decidedly acid, but avoid an excess of the acid. Suspend the beaker in a water bath and keep the water in the bath at the boiling temperature. At the expiration of 30 minutes, if, by transmitted light, the urine is clear between the flakes of coagulated albumen, the precipitation is complete. If, however, the urine is cloudy, a small quantity of acetic acid is added, the urine stirred, and the heat continued, when the separation of albumen in flakes will take place. Filter through a filter, having been dried at 110° C. between watch glasses and cooled in a desiccator and weighed. The albumen having been transferred to the filter, is washed with water. As the filtering and washing are likely to require several hours, a filter pump or aspirator bottle may be employed with advantage, the filter having the support of a platinum cone. The washing is continued until no cloudiness is produced when tested with a solution of silver nitrate and some nitric acid. Having been washed with water, wash with about 50 cc. absolute alcohol, followed by about the same quantity of ether. Any fat present is removed by the alcohol and ether, and the water is so far removed as to facilitate the drying. The funnel is covered with paper or a glass plate and placed upright in an air bath and heated gradually until the paper and precipitate are somewhat dry, when the filter, with the precipitate, is placed between the watch glasses employed before. The heating in the air bath at 110° C.

is continued until the weight becomes constant, which is ascertained by heating two hours, cooling in a desiccator and weighing, repeating the process until the weight becomes constant. The difference in weight caused by the precipitate is taken as the weight of albumen, except in case the urine contains much albumen; when the filter paper and precipitate are ashed and the ash weighed in a platinum crucible. By subtracting the weight of the ash from that of the precipitate, the remainder is the weight of albumen, or, instead of ashing, 50 cc. urine may be taken and 50 cc. water added before acidifying and heating. The albumen, when dry, should not exceed 0.3 grm. in weight; if less, the quantity of inorganic matter present is very small.

GLOBULINE.

HAMMARSTEN'S METHOD.

For outline of the method for separating globuline from the urine, refer to page 47. In separating globuline from albuminous urine with magnesium sulphate, the urine is rendered as nearly neutral as possible without precipitating calcium and magnesium phosphates. For this purpose dilute acetic acid and a weak solution of sodium carbonate are used. If the urine is highly colored and the globuline not in very small quantity, it is diluted with water 25 or 50 cc. urine with 75 or 50 cc. water. If the quantity of globuline is too small to admit of dilution, and the urine is highly colored, surround the flask or beaker containing it with ice broken in small fragments, and when the temperature of the urine has been reduced to 2° or 3° C. several hours, the urates may precipitate, carrying with them much of the coloring matter of the urine. Filter, while cold, through a dry folded filter paper. To 100 cc. urine or urine and water, if diluted in a beaker, add 80 grms. pure powdered magnesium sulphate. Stir continually until the urine becomes saturated; avoid, however, the formation of foam. Add more of the magnesium sulphate, if necessary, and let stand 24 hours, stirring occasionally. Globuline precipitates while serum-albumen, hemialbumose and peptone remain in solution. Filter through a small filter paper free of ash, having been dried at 110° C. in an air bath between two watch glasses, and weighed after having cooled in a desiccator.

Before filtering, the filter is wet with a saturated solution of magnesium sulphate. Wash the precipitate with a saturated solution of magnesium sulphate until the wash water, when acidified with acetic acid, remains clear by boiling (absence of albumen), when the funnel is placed upright in an air bath—covered to protect from dust—and heated to 110° C. at least 3 hours. By the heat the globuline becomes insoluble. Let cool and wash with hot water until the wash water, when tested with a solution of barium sulphate, produces no cloudiness (absence of $MgSO_4$), after which the precipitate is washed with absolute alcohol two or three times, and as many times with ether. Place the filter and precipitate between the watch glasses. Dry at 110° C., and after cooling in a desiccator, weigh. Repeat the process until the weight becomes constant. The increase in weight caused by the precipitate having been noted, the filter and precipitate are ashed in a weighed platinum crucible, and the weight of the ash is subtracted from the weight of the dry precipitate; the remainder is the weight of globuline in the quantity of urine employed.

POHL'S METHOD.

Render the urine neutral by employing dilute acetic acid and a weak solution of ammonium hydrate; filter, if necessary, and mix in a beaker 100 cc. of the filtrate—or urine, if not filtered—with 100 cc. of a saturated solution of neutral ammonium sulphate, and after standing one hour, separate the precipitate—globuline—by filtering through a weighed filter, having been dried at 110° C., and wash the precipitate with a mixture of 1 volume saturated solution of ammon. sulphate and 1 volume water, until the wash water yields no precipitate or cloudiness when tested with a solution of potassium ferrocyanide acidified with acetic acid. Place the funnel upright in an air bath and heat three hours at 110° C. to render the globuline insoluble in water. When cool, wash with hot water until the wash water is free of ammon. sulphate, known by yielding no turbidity when tested with a solution of barium chloride. The washing is then continued with 100 cc. alcohol, followed by an equal volume of ether. Dry the filter and precipitate between the watch glasses, employed before at 110° C., and having cooled in a desiccator, weigh. This process is repeated until the weight is constant.

Having ascertained the weight of the precipitate, ash it with the filter in a weighed platinum crucible, and subtract the weight of the ash from the weight of the precipitate, the remainder is the weight of globuline in 100 cc. urine.

HEMIALBUMOSE.

There is no method for determining the exact quantity of hemialbumose in urine, but the quantity within narrow limits is determined. To prepare urine for the estimation, separate the albumen by adding to 500 cc. urine ⅙ its volume (85 cc.) of a saturated solution of common salt—sodium chloride—and if the urine is not decidedly acid in reaction, dilute acetic acid is added until it is acid. Heat to the boiling temperature and filter. To precipitate hemialbumose in the filtrate add an equal volume of a saturated solution of common salt, and after stirring filter through a dry weighed filter paper, free of ash, and having brought the precipitate on the paper, wash it with a saturated solution of common salt.

Dry the filter with precipitate at 110° C. until the weight is constant, and note the increase in weight caused by the precipitate and some common salt. Ash the filter and precipitate, and weigh the ash in a porcelain or platinum crucible, and subtract the weight of the ash from the weight of the precipitate: the remainder is the approximate weight of hemialbumose in 500 cc. urine.

Instead of weighing the precipitate, it may, without drying, be dissolved in water, and the quantity determined, according to Salkowski, by means of a polariscope. The specific rotation of hemialbumose is — 75°. For the use of the polariscope, refer below. By employing a tube 10 cm. long, the equation for the percentage is— $75 : 100 :: \overset{\text{degrees}}{\text{read}} : x$. If the solution of hemialbumose examined occupies the volume of 500 cc., no further calculation is required, but if 250 cc., then the per cent. found is divided by 2.

PEPTONE.

HOFMEISTER'S METHOD.

This method is based on the property peptone has, when in solution, of changing the plane of vibration of a ray of light having been polarized. For outline of description of polariscope,

refer below. To prepare the urine for examination, it is decolorized by treating 80 cc. of the urine in a 100 cc. graduated flask with a few drops of a solution of neutral lead acetate, when the flask is filled with water to the mark. Shake well and filter through a dry filter paper into a dry flask, and examine the filtrate with a polariscope. The specific rotation of peptone is — 63.5°. The calculation is made by the equation 63.5 : 100 : : degrees read : x, if a tube 10 cm. long be employed. From the per cent. or weight of peptone found in 100 cc. of the solution examined, the quantity in 100 cc. urine is determined by multiplying by $\frac{1}{8}$ or 1.25, as 100 cc. of the solution corresponds to 80 cc. urine. This method does not provide for the presence of albumen or hemialbumose in the urine, as these bodies also change the plane of vibration of polarized light. If present they are separated (Hofmeister), by adding to 80 cc. acid urine a solution of sodium carbonate, until the urine is slightly acid in reaction, and in case the urine is alkaline dilute acetic acid is added until the urine is acid, yet avoiding an excess. The urine is then treated with 10 cc. of a saturated solution of sodium acetate, when a solution of ferric chloride is added until the urine becomes somewhat red in color. Boil a short time, let cool, and with water dilute to the mark 100 cc., and having mixed well by shaking, filter through a dry filter into a dry flask or beaker. The filtrate when examined with the polariscope should not yield a precipitate with a solution of potassium ferrocyanide and acetic acid.

REMARKS.

Of the great number of methods for the estimation of albumen in the urine, however ingenious some of them are, there are none which lead to more accurate results than that of Scherer, although the drying and filtering by this method require time and close attention. The methods of estimating hemialbumose and peptone are not exact.

SUGAR.

FEHLING'S METHOD.

For the facts on which this method is based, refer to page 50.

PREPARATION OF SOLUTIONS.

SOLUTION OF COPPER SULPHATE.

Commercial copper sulphate is purified by dissolving as much of the salt in hot water as possible, and filtering while hot into a beaker, and let crystallize by standing in a cool place. The mother liquid is poured off and the crystals collected in a funnel in the neck of which there is some spun glass, to prevent the crystals from passing through when they are washed with a small quantity of cold water. Dissolve again in hot water and continue as before. The crystals are dried by pressing several times between porous paper. To prepare the solution, weigh on a balance of precision 34.639 grms. of the copper sulphate. Introduce the salt into a 500 cc. graduated flask and dissolve in some water and fill with water to the mark. Mix well by shaking.

SOLUTION OF SODIUM POTASSIUM TARTRATE AND SODIUM HYDRATE.

Dissolve 173 grms. sodium potassium tartrate in about 300 cc. warm water in a 500 cc. graduated flask. If the solution is not clear, filter, and to the solution add 50 grms. sodium hydrate, and when dissolved fill with water to the mark, but not until the temperature is reduced to about 17° C.

FEHLING'S SOLUTION.

Fehling's solution is prepared by mixing an equal volume of the solution of copper sulphate and the solution of sodium potassium tartrate and sodium hydrate; but as Fehling's solution is liable to change, the solutions should be prepared for immediate use and, therefore, in less quantities.

Before using Fehling's solution, test it by heating to the boiling temperature in a flask 10 cc. of the solution diluted with about 40 cc. water, and after cooling and standing several minutes, if no Cu_2O is found remaining on the bottom of the beaker when the solution is decanted, there is no reducible substance in the solution. If Fehling's solution has been prepared with care, there is no necessity of testing its strength with a solution of grape sugar. 1 cc. of Fehling's solution = 5 milligr. diabetic or grape sugar.

THE TITRATION.

The titration is made with the urine instead of the reagent. 10 cc. Fehling's solution is introduced into a 100 cc. flask with a pipette. For accurate results, sugar in the urine should be in such quantity that between 6 and 10 cc. of the urine would be required to reduce the CuO in 10 cc. of Fehling's solution, but as there is generally more sugar in diabetic urine, it requires dilution with water. Urine containing but little coloring matter, and by qualitative tests yields reactions indicating the presence of much sugar, may be diluted from 1 to 10 volumes without a preliminary estimation. On the other hand, if the indications are that the amount of sugar is not great, preliminary estimations are made. A burette having been cleaned and dried, is filled with urine, and the 10 cc. Fehling's solution in the flask is diluted with 40 cc. water placed on a wire gauze and heated with a gas or spirit lamp until it begins to boil, when 0.5 cc. of the urine is added from the burette, a yellow or red precipitate will form. After shaking, let the precipitate settle. If sufficient urine has been added to reduce the CuO, the blue color of the solution will have disappeared, as seen by the surface of the fluid, when at an angle of about 45°, with a white background. If the blue color is perceptible, heat again to the boiling point, and add 0.5 cc. urine, and if the blue color still remains after settling, repeat the titrations until the blue color disappears. Having made one or two preliminary titrations, dilute the urine if necessary. In diluting use round numbers and measure in a graduated 100 cc. cylinder; for example, 3 cc. urine decolorizes 10 cc. of Fehling's solution. Into the cylinder introduce 30 cc. of the urine and with water fill to the mark of 90 cc.; the dilution is 1 to 3. Theoretically, 10 cc. of Fehling's solution would require 9 cc. of the diluted urine. Titrations may now be made, adding 8.6, 8.8 and 9 cc. of the diluted urine, and if the cupric oxide is not all reduced, titrations are made, adding an increased quantity of 0.2 cc. until complete reduction takes place. After diluting the urine, time is saved by heating three or four flasks containing Fehling's solution with water at the same time, and by employing a 50 cc. burette containing the diluted urine, and keeping a record of the quantity of urine added to the contents of each flask.

Instead of depending on the disappearance of the blue color of Fehling's solution, after settling, a small quantity of the fluid is decanted into a test tube and heated with 0.1 or 0.2 cc. of the urine, and if CuO is present a yellow or red precipitate will form, or, as has been recommended, a small quantity of the fluid may be filtered, the filtrate acidified with acetic acid and tested with a solution of potassium ferrocyanide; if CuO is present, a red precipitate or coloration takes place, but by boiling urine with sodium hydrate ammonia is formed, which dissolves a small quantity of cuprous oxide, and by exposure to the air during the process of filtering it oxidizes, forming cupric oxide; consequently, this method of determining when reduction of CuO has taken place is not satisfactory.

CALCULATION.

Suppose the urine was diluted from 1 to 9 and that 8.1 cc. of the diluted urine was required to reduce the copper oxide in 10 cc. Fehling's solution. The urine in 8.1 cc. of the diluted urine is $\frac{1}{9}$ of 8.1, which is 0.9 cc., hence there is in this amount of urine sufficient sugar to reduce the cupric oxide in 10 cc. Fehling's solution. 1 cc. Fehling's solution corresponds to 0.005 grm. sugar, and 10 cc. to 0.050 grm., therefore in 0.9 cc. urine there is 0.050 grm. sugar, and in 100 cc. urine 5.555 grms. sugar (0.9 : 0.050 :: 100 : x).

FEHLING'S METHOD MODIFIED BY PAVY.

In the estimation of sugar in the urine by Fehling's method, it is often very difficult to determine the point, in titrating, when the cupric oxide is reduced, as cuprous oxide remains suspended. To obviate this difficulty, Pavy's solution may be employed and the titration carried on in the absence of air. In this solution there is ammon. hydrate, by which the cuprous oxide or hydrate is held in solution, so that complete reduction of the cupric oxide is known by the solution losing its blue color. As cuprous oxide oxidizes readily by exposure to air, especially when dissolved in water by means of ammonia, provision is made for the exclusion of air while the reduction takes place. Pavy's solution has $\frac{1}{10}$ the reducing effect of Fehling's solution.

PAVY'S SOLUTION.

Introduce 120 cc. of Fehling's solution (for the preparation of which refer to page 171) into a 1000 cc. graduated flask containing 300 cc. ammon. hydrate, sp. gr. 0.80; when 140 grms. sodium hydrate is added, and when solution has taken place and the temperature reduced to about 17° C., fill with distilled water to the mark, mix well by shaking.

THE TITRATION.

To prevent the access of air to the fluid titrated, a 200 cc. round bottom flask is provided with a cork having two holes, through one of which passes the dropper of the burette containing the urine, and through the other hole a bent glass tube passes which is connected with a chlor-calcium tube, filled with fragments of pumice saturated with dilute sulphuric acid. Introduce 50 cc. Pavy's solution into the round bottom flask, and when the solution is heated to near the boiling point, urine is added from the burette, but, as reduction takes place more slowly than with Fehling's solution, care is taken not to boil the solution actively, or the ammonia may be driven off, the cuprous oxide precipitated and the solution lose much of its blue color before the cupric oxide has been completely reduced. The cupric oxide is reduced when the solution becomes colorless or slightly yellow in color. The difficulty encountered by the escape of ammonia before reduction takes place is obviated by introducing the dropper of a separating funnel into the flask through a hole in the stopper, and during the titration adding small quantities ammon. hydrate; but this precaution will not be found necessary if ammon. hydrate of the sp. gr. 0.80 be employed in preparing the solution, and nearly as much urine as is required for complete reduction of the CuO be added at once and the solution heated a few moments to the boiling point. In the employment of Pavy's solution urine is diluted if necessary, so that 7 to 10 cc. urine contains sugar in quantity to reduce the cupric oxide in 50 cc. Pavy's solution.

CALCULATION.

The quantity of sugar to reduce the CuO in 50 cc. Pavy's solution is 0.025 grm., taking which into account the calculation is the same as by the employment of Fehling's solution.

ROBERTS' METHOD.

This method rests on the fact that by fermentation of sugar in the urine the specific gravity of the urine becomes less according to the quantity of the sugar present. The urine is filtered if not clear. The specific gravity of the urine is determined by means of Sprengel's picnometer, which is provided with a thermometer and capillary tube. With this apparatus the specific gravity of the urine is determined with greater accuracy than with an ordinary picnometer or urinometer. 300 or 400 cc. of the urine, sp. gr. having been determined, is introduced into a flask, capacity 1000 cc., and some yeast cake having been well washed with distilled water is mixed with the urine. Place the flask in a vessel containing water, a large water bath for example, so that it is surrounded by water, and keep the temperature of the water at 20 to 25° C. about 30 hours, with a microcosmic lamp. At the end of the process the yeast cells will have settled and the fluid be nearly clear. Filter through a dry filter paper into a dry flask, and bring the filtrate to the temperature at which the urine was when the specific gravity was determined, and ascertain its specific gravity with Sprengel's picnometer, taking care that the temperature be the same in both determinations.

CALCULATION.

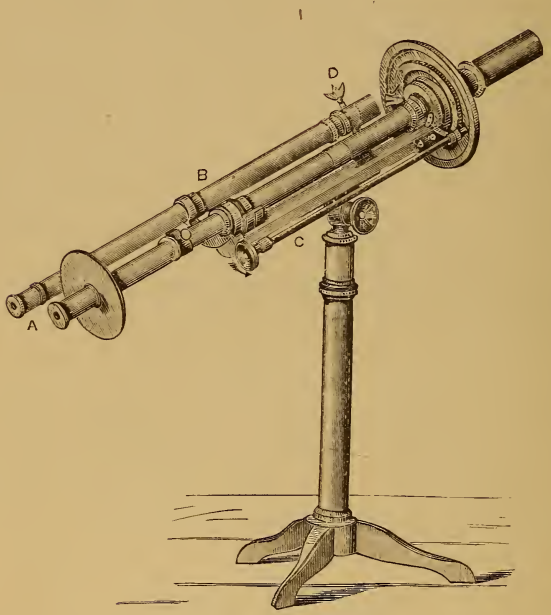
Multiply the difference between the specific gravity of the urine before and after fermentation by 230, the product of which is the per cent. of sugar in the urine. If, however, the per cent. is less than 0.5, multiply by 219 instead of 230.

THE OPTICAL METHOD.

In almost all saccharimeters there are two Nicol's prisms, one of which polarizes the light, and the other acts as an analyzer. A ray of light entering the polarizer is divided in two parts vibrating at right angles to each other, but the construction of the prism is such that the ray vibrating in one plane is absorbed while the other passes through. Light, therefore, which passes through and vibrates in one plane is polarized. Now, if the second prism or analyzer is related to the first so that their oblique ends are parallel, the polarized ray will pass through it without

obstruction; but if rotated on its axis, the light passing through gradually becomes less until the rotation reaches 90°, when the light is cut off. From 90°, if the rotation be continued, light begins to pass, and the quantity increases until 180° is reached, when the polarized light again passes without obstruction, and by continuing the rotation from 180°, the light passing becomes less bright until the rotation is 270°, when no more light passes, and from this degree the light appears as the rotation takes place and passes without obstruction when the rotation is 360°, or the

FIG. 36.



original position of the prism. If the prisms are so related that the polarized light passes without obstruction, and there be placed between them a solution of grape or diabetic sugar in a glass tube, having its ends closed by glass plates, the plane of vibration of the light is rotated by the sugar so that when it falls on the second prism it is partly obstructed in its course; however, by turning the latter on its axis, a degree of rotation is reached when the light passes through it. The plane of polarization is turned according

to the amount of sugar in solution, and the length of the tube containing the solution, but the difficulty of determining at what degree of rotation the greatest amount of light is transmitted is very great; hence, in nearly all saccharimeters means are employed by which it is at once determined when the light reaches the eye in the greatest quantity, one of the prisms being in connection with the arc of the circle or disc, which is graduated in degrees and minutes, so that when the prism is turned, either the disc or vernier is likewise turned. It is not necessary to enter here into a consideration of the devices employed in the various saccharimeters in use.* For exact results, the apparatus of either Wild or Laurent is preferable, as the use of either does not depend on the ability to note differences in tints of color; hence errors cannot arise from color blindness. In either apparatus the sodium light

FIG. 37.

is employed. It is produced by sodium chloride, supported by a loop of platinum wire in a Bunsen's flame. Laurent, however, has constructed a special lamp — Laurent's Eolipile — for the production of the sodium light. The general arrangement of Wild's saccharimeter is represented by Fig. 36. In this apparatus the relationship of the two Nicol's prisms is known by the disappearance of black horizontal lines in the centre of the field of vision, as seen by *b*, Fig. 37. Crossing at acute angles are two lines, which remain distinctly in view at all times if the telescopic part of the polariscope is adjusted, which is near the eye piece. The graduated disc and the Nicol's prism by which the light is polarized are turned by means of the rod, C, the distal end of which is geared with the disc. The markings of the disc in

* For an exhaustive treatment of the subject, the student is referred to Dr. Landolt's Handbook of the Polariscope and its Practical Applications, translated. MacMillan & Co.

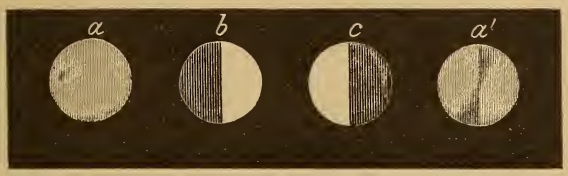
degrees are read by means of the telescope, B, and they are made visible by a special lamp, D. Before estimating sugar in urine, the telescope is adjusted so the lines on the scale are well defined. This is accomplished by moving the ocular of the telescope forward or backward until the markings are plainly visible. The sodium flame having been placed in the axis of the saccharimeter, and one of the tubes, empty or filled with distilled water, placed in position, the telescopic part of the polariscope, A, is adjusted so the cross lines are distinctly seen when the graduated disc is turned by the rod, C, until the horizontal lines disappear from the centre of the field *b*, Fig. 37. The instrument is adjusted if the vernier is found at 0, 90, 180 or 270°. In determining when the horizontal lines disappear, the cross lines serve the purpose of ascertaining if the telescopic part of the polariscope, A, has remained in adjustment, or if the eye has changed in its accommodation; in either case the telescopic part is adjusted so the cross lines are sharply defined, and if necessary the graduated disc is again turned until the horizontal lines disappear from the centre of the field of vision If a variation is met, that is, if the vernier is not at 0 when the horizontal lines disappear from the central portion of the field of view, tests are made in the different quadrants of the circle, and in making estimations the variation is taken into account.

In Laurent's apparatus the blue and violet rays of the sodium light are separated by the light passing through a plate of potassium bichromate. Half way across the path of the light, having been polarized, is a quartz plate of uniform and definite thickness. It is by means of this plate that, when the prisms sustain certain relationships, the half of the field of view crossed by the quartz plate is dark, while the other half is light, and by other relationships of the prisms both halves of the field of view are equally light. To adjust the instrument, the small cog wheel geared with the large graduated disc is turned to the right or left until the field of vision becomes equally light, and the margin of the quartz plate disappears, as shown by *a*, Fig. 38. If the 0 of the vernier corresponds to that of the graduated disc, the instrument is adjusted.

To estimate sugar in the urine it should be clear, and if not clear, it is filtered. Sugar in highly-colored urine cannot well be

estimated with a saccharimeter, and especially is this true with Laurent's apparatus. However, diabetic urine is generally free of much coloring matter, except in case of fever. To remove excess of coloring matter, shake the urine in a flask with a small quantity of pulverized neutral lead acetate and filter. To avoid diluting the urine, the flask should be dry and the filter not moistened with water before filtering. In filtering or decolorizing urine, sufficient quantity is taken to examine with tubes of different lengths. Before filling the tubes, the water in them is removed by rinsing with the urine several times. The presence of water not only dilutes the urine, but, unless it is intimately mixed with the urine, it interferes with the passage of light. The effect which albumen has in changing the plane of vibration of polarized light is so inconsiderable that, when in small quantity in the urine, its removal is not necessary, but if in considerable quantity it should be removed; consequently, tests for albumen are made before

FIG. 38.



estimating sugar with a saccharimeter. To separate albumen, 9 volumes of urine (Hofmeister) are mixed with 1 volume of a solution of sodium phosphortungstate which has been rendered strongly acid with hydrochloric acid. Having shaken the mixture in a bottle or flask, filter through a dry filter into a dry flask; the tube, 10 cm. long, having been filled with urine and closed, to the exclusion of air, is placed in position, the sodium light having been brought in the axis of the saccharimeter and the vernier at 0. In Wild's apparatus the graduated disc is turned to the left (in opposite direction to that of the hands of a clock) until the horizontal lines disappear from the centre of the field of view. As in adjusting the apparatus, attention is directed to the cross lines, and if they are indistinct, the telescopic part of the polariscope, A, is adjusted before proceeding. In Laurent's apparatus the vernier is moved from above to the right, as the hands of a clock

(this motion is imparted by turning the cog wheel to the left), to reach the degree at which the light of each half of the field of vision becomes equally bright, and the perpendicular margin of the quartz plate disappears from view. As a control of the result reached by use of the tube 10 cm. long with either apparatus, the tube 20 cm. long is employed, and the number of degrees rotated should be twice that of the first; or dilute 1 vol. urine with 1 vol. water, and having mixed well examine with the tube 10 cm. long; the number of degrees rotated should be one-half that with the urine before dilution.

The calculation of the per cent. of sugar in urine from the number of degrees rotated with the tube 10 cm. long is quite simple. Multiply the number of degrees of rotation by 100 and divide by 53.1 (specific rotation of diabetic sugar), the quotient is the per cent. of sugar in the urine.

REMARKS.

As normal urine contains substances which reduce copper oxide, the results of Fehling's method, or Pavy's modification of Fehling's method, are 0.2 to 0.4 per cent. too high. Roberts' method yields good results, and in many respects is preferable in estimating sugar in urine to that of Fehling or Pavy. It has the advantage of not requiring much apparatus. Instead of employing Sprengel's picnometer to determine the specific gravity, a urinometer may be used, when the results will be approximative. The results obtained by the Optical Method are the most accurate. If, in estimating sugar in the urine by Roberts' method and also by the Optical Method, the results are considerably higher by the former than by the latter, there is reason to believe that fruit sugar is present. Although fruit sugar has the same molecular constitution as diabetic sugar, and it undergoes fermentation and reduces CuO, yet, when in solution, it changes the plane of vibration of polarized light to the left instead of the right; as diabetic sugar, therefore, the results of the optical method are too low. In diabetic urine, however, this form of sugar is seldom found.

APPENDIX.

TABLE 1.—NITROGENOUS COMPOUNDS, EXCEPT UROBILIN, IN NORMAL URINE.

Constituent.	Per cent. of Nitrogen.	Relative number of parts of each constituent.	Average number of grammes of each constituent excreted in 24 hours by adult of average wt., etc.	Number of grammes nitrogen excreted in 24 hours, calculated from the per cent. of nitrogen in each compound.
Urea	46.66	1.0	31.0	14.464
Uric Acid . .	33.33	0.01938	0.6	0.199
Ammonia . . .	82.35	0.02257	0.7	0.576
Kreatinin . . .	37.26	0.02257	0.7	0.260
Hippuric Acid .	7.87	0.01129	0.4	0.031
Indican	5.57	0.00035	0.011	0.0006
Xanthin . . .	36.85	——	0.0056	0.00206

Total number grms. nitrogen excreted in 24 hours by adult of average weight, etc.	15.53266
Average number of grms. nitrogen of urea excreted in 24 hours by adult of average weight, etc.	14.4640
Average number of grms. nitrogen of compounds other than urea excreted in 24 hours by adult of average weight	1.06866
Per cent. of nitrogen in combination in urea of the total quantity excreted	93.12
Per cent. of nitrogen in combination in other compounds than urea of the total quantity (100 per cent.) excreted	6.88

TABLE 2.—(FROM ZUELZER'S "SEMIOLOGIE DES HARNS.")—NUMBER OF PARTS OF NITROGEN AND OTHER CONSTITUENTS OF ELEMENTS OF FOOD IN 1000 PARTS.

Element of Food.	Nitrogen.	P_2O_5.	H_2SO_4	K.	Na.	MgO.	CaO.	Cl.
Muscular tissue of the ox. . .	34.6	4.45	5.6	3.6	0.58	0.29	0.16	0.691
Calf	35.7	3.73	——	2.19	0.43	0.15	0.13	0.5
Horse	30.19	4.85	8.2	2.8	0.71	——	0.107	0.202
Brain (all parts of) of the ox.	——	7.416	trace	3.26	1.32	0.135	0.36	0.514
Milk (human) .	5.4	0.437	0.07	0.7	0.09	0.02	0.431	0.437
Milk (cow) . .	3.81	2.1	0.395	0.875	0.47	0.299	1.864	0.75
Wheat.	20.8	7.9	0.12	4.3	0.29	2.00	0.60	0.03
Oats	19.2	6.2	0.49	3.6	0.44	1.9	1.00	0.15
Potatoes . . .	3.2	1.6	0.85	4.8	0.12	0.4	0.20	0.29

TABLE 3.—(FROM ZUELZER'S "SEMIOLOGIE DES HARNS.")—RELATIVE NUMBER OF PARTS OF CONSTITUENTS OF CERTAIN ANIMAL AND VEGETABLE BODIES FOR 100 PARTS OF NITROGEN WHICH THEY CONTAIN.

	P_2O_5.	H_2SO_4	K.	Na.	MgO.	CaO.	Cl.
Muscular tissue (human). .	12.1	23.1	9.0	4.5	1.1	0.6	——
Muscular tissue (horse). .	15.7	24.0	9.1	2.3	1.4	0.4	0.5
Muscular tissue (ox) . . .	12.8	16.7	10.4	1.6	1.5	0.6	1.9
Muscular tissue (calf). . .	10.4	——	6.1	1.1	0.4	0.3	1.4
Brain (average)	44.0	0.7	21.0	8.7	1.1	0.6	2.6
Blood from all parts of body.	——	1.5	3.5	6.0	1.0	3.0	6.0
Milk (human).	13.4	2.0	18.8	4.4	1.4	10.7	12.9
Milk (cow)	55.1	10.3	23.4	12.3	7.9	49.0	19.7
Wheat	38.0	0.57	20.6	1.3	9.6	2.8	0.1
Oats	37.5	2.5	18.7	2.2	9.8	5.2	0.7

TABLE 4.—(FROM ZUELZER'S "SEMIOLOGIE DES HARNS.")—INDICATING CHANGES IN THE CONSTITUTION OF THE URINE BY THE INGESTION OF DIFFERENT KINDS OF FOOD. NUMBER OF PARTS FOR 100 PARTS OF NITROGEN EXCRETED.

	P_2O_5.	H_3SO_4.	MgO.	CaO.	K.	Na.
Blood . . .	11.9	19.0	1.0	0.4	16.6	11.2
Beef	11.0	17.1	0.4	0.2	9.6	4.1
Horse flesh .	15.4	16.8	0.5	0.7	——	——
Kidney . .	23.0	25.6	0.5	0.4	——	——
Liver, cooked	26.3	18.8	0.4	0.5	10.8	10.8
Liver, fresh .	31.3	24.9	——	0.4	12.4	6.5
Brain . . .	33.1	10.8	0.08	0.4	——	——
Brain . . .	30.8	25.5	0.7	0.8	22.7	8 8
Milk	21.3	10.1	0.8	1.2	34.1	38 9

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